Actions of the rubrospinal tract in the cervical spinal cord of the rat

Sarah Al-Izki

Institute of Neurology

University College London

Submitted for the degree of Doctor of Philosophy (PhD)

April 2007

Supervisor: Dr. Peter Kirkwood

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

Abstract

This thesis describes the electrophysiological and anatomical distribution of the projections from the rubrospinal tract (RST) in the cervical spinal cord of the rat, in control animals and animals with a dorsal column lesion. It also describes the behavioural changes following the lesion.

The RST originates from the magnocellular region of the red nucleus (RNm), from where its axons project to the contralateral spinal cord, mostly in the dorsolateral funiculus (DLF). Previous work has shown that the RST is implicated in precise limb movements. It has also been demonstrated that where the corticospinal tract (CST) is impaired, the RST is capable of motor control and will compensate for the damage to the CST.

In terminal experiments, electrical stimulation of the RNm elicited two orthodromic descending volleys recorded from the contralateral DLF. The latency of the early one indicated that it is produced by direct activation of fibres in or close to the RNm. Temporal facilitation demonstrated that the second volley is elicited by synaptic excitation of RNm neurons. Postsynaptic responses related to the second volley were seen only in the intermediate zone of the contralateral spinal cord. In animals with a dorsal column lesion, the distribution of RST actions is similar to that of the controls.

Functional recovery in lesioned animals was assessed using three behavioural tests: pellet retrieval, cylinder, and the sticker removal test. The cylinder and sticker removal tests have both shown to be ineffective in providing important information on the recovery. However, the pellet retrieval test, which requires skilled motor control, has enabled correlation of recovery to size of lesion. The results obtained demonstrate that lesion to dorsal columns alone has little effect on the success rate.

The anatomical and electrophysiological experiments provide information on the distribution of RST actions, which will be correlated in the thesis with the behavioural results. Evidence for plasticity will be discussed.

Contents

List of figures

List of tables

Abbreviations

BDA:	Biotinylated dextran amine.
BDNF:	Brain-derived neurotrophic factor.
CNS:	Central nervous system.
CNTF:	Ciliary neurotrophic factor.
CST:	Corticospinal tract.
DC:	Dorsal columns.
DLF:	Dorsolateral funiculus.
EPSP:	Excitatory post-synaptic potential.
FP:	Field potential.
GAP-43:	Growth associated protein 43.
IPSP:	Inhibitory post-synaptic potential.
ML:	Medial lemniscus.
NT3:	Neurotrophin-3.
PNS:	Peripheral nervous system.
NGF:	Nerve growth factor.
NSFCs:	Neurosphere forming cells
RN:	Red nucleus.
RNm:	Magnocellular red nucleus.
RNp:	Parvocellular red nucleus
RST:	Rubrospinal tract.
SCI:	Spinal cord injury.
vCST:	Ventral corticospinal tract.

Acknowledgments

I would like to acknowledge the following people for their help and support during the course of my Ph.D. First, I would like to thank my lab; my deepest gratitude goes to my supervisor Dr. Peter Kirkwood. With his vast knowledge and enthusiasm he has provided advise, guidance, and encouragement during this study. I extend my sincere thanks to Dr. Manuel Enriquez Denton, for the invaluable training that he provided. I would like to thank Roseanna Smither, for all her help during my Ph.D., in assisting in surgeries and experiments, behavioural training, histology, and so much more. And finally, from my lab, I would like to thank Anoushka de Almeida for her support and encouragement.

I wish to extend my thanks to my second supervisor Professor Roger Lemon and his lab for all their help and advice, especially Gita Prabhu.

I would like to thank to Dr. Linda Greensmith for her greatly appreciated advice, and also to members of her lab, above all Joanna Riddoch-Contreras.

I would like to extend my deepest thanks to many other people in the Sobell department who have made this possible with their advice and kind help; Dr. Chris Seers, for constant IT support and great friendship, Ed Bye, Victor Baller, Kully Sunner, Debbie Hadley and Molly Cureton.

My warmest thanks go to my parents for their encouragement and support over the miles. And finally, I am forever indebted to Dave Walshe for his understanding, patience, and endless support. I thank him for his trust and great belief in me, and for always being there for me. Thank you.

Chapter 1. Introduction

Spinal cord injury (SCI) causes severe debilities and till this day remains incurable. A number of spinal cord injury animal models have been developed to assess regeneration, plasticity, and functional recovery. It is generally said that contusion and compression models of SCI are of more clinical relevance because the histopathology of these injuries resemble SCI in humans the most. However, tract specific lesions are often used to assess specific pathways within the spinal cord. This allows a more precise investigation of defined pathways and the areas of influence they have on the spinal cord and their physiological role within the central nervous system (CNS) and also the assessment of their regeneration and plasticity following a SCI.

In this thesis, the connectivity of the rubrospinal tract (RST) in normal animals and in animals with a lesion to the dorsal columns including the corticospinal tract (CST) was investigated. This was addressed using physiological and anatomical studies to assess the areas of influence of the RST in the caudal cervical spinal cord, together with behavioural studies to assess the level of spontaneous functional recovery. This first chapter provides background information on the RST and the CST but mainly focuses on the RST, covering the anatomical and physiological literature. Other topics covered include the factors that affect SCI and repair, a brief summary of the anatomy of the spinal cord of the rat, the C3-C4 propriospinal system, and the corticospinal tract.

1.1 Spinal cord injury

The effect of a SCI depends on the type and level of injury. SCI's can be classified into complete or incomplete injuries. A complete injury means that there is no sensation or voluntary movement below the level of the injury. An incomplete injury means that there is some sort of functionality below the level of the injury, which could be sensory or motor or both. Cervical injuries result in quadriplegia while thoracic level injuries or below result in paraplegia. Higher cervical injuries could be as severe as requiring the person to use a ventilator. A C5 injury usually results in a person with only shoulder and biceps control, a C6 injury allows wrist control but no hand function. C7-T1 injuries may have arm control but not complete control of hands and fingers. People with thoracic level injuries usually have complete hand control. Unlike lower thoracic injuries, upper thoracic injuries results in poor trunk control. As for lumbar and sacral injuries, these result in a decreased control of hip flexors and legs. However, these are not the only affects of SCI; people with SCI usually experience dysfunction of the bowel and bladder, reduced sexual function, low blood pressure and the inability to regulate it, reduced control of body temperature, and chronic pain.

Over the decades, many researchers have dedicated their time to investigating SCI, the biology and biochemistry underlying the injuries and the molecular targets for therapeutic intervention following SCI. One of the many issues addressed during research into spinal cord repair is coaxing lesioned axons to regenerate and make functional connections with their targets. Other issues include reducing secondary damage and to translate the knowledge obtained in the laboratory into human clinical trials. However, regeneration is not the key to recovery following SCI. Most SCIs are partial and result in the sparing of tissue and axonal pathways and therefore an

important strategy is directed towards these spared pathways, to optimise their function, to explore their plastic behaviour, and to investigate whether spared pathways may compensate for damaged ones. Understanding the plastic behaviour of these pathways and targeting them to optimise their sprouting and plasticity is an important issue for SCI and repair, and therapeutic interventions targeted at plasticity may be a crucial step towards the achievement of spinal cord repair and functional recovery.

Animal models of SCI are crucial in studying its various aspects. In this way, the regenerative capacity of damaged axons and/or the sprouting of spared ones can be studied in an environment that mimics that of the human spinal cord. To obtain the maximum amount of information from experiments, and also to minimise the number of animals used, anatomical studies must be combined with electrophysiological and behavioural assessments. Electrophysiological assessment of fibres that have regenerated or sprouted is an important tool for assessing whether they make functional connections and is also a powerful tool to explore the areas of influence of these pathways within the spinal cord. Behavioural studies are vital in exploring the recovery of motor and/or sensory functionality following SCI.

1.2 Factors influencing spinal cord injury and repair

There are two major events that occur following a SCI, first, enlargement of the lesion area due to secondary cell death. Second, a cascade of cellular reactions occurs in the surrounding central nervous system (CNS) including the invasion of inflammatory cells and reactive astrocytes, and the formation of cavities and glial scars. Some therapeutic interventions for SCI are aimed at preventing secondary cell death and interfering with the cellular events that occur following SCI.

1.2.1 Inflammatory Reponses

Following SCI, an inflammatory reaction occurs and many factors are recruited including neutrophils, monocytes, and glial cells. Following SCI and in the peripheral nervous system (PNS), a large number of macrophages are recruited which secrete proteases and engulf myelin and axonal debris degrading the distal lesioned end of the axon. The macrophages also secrete factors that promote axonal growth and stimulate Schwann cell proliferation. However, in the CNS, the number of inflammatory cells recruited is much lower than that in the PNS. These inflammatory cells are localised to the injury site and their levels peak at a later stage compared to the PNS (Perry *et al.*, 1987;Lawson *et al.*, 1994;Hirschberg & Schwartz, 1995).

Inflammatory responses may have both detrimental and beneficial roles in the CNS and have been linked to secondary tissue damage, progressive cavitation, and glial scarring (Dusart & Schwab, 1994). Neutrophils and macrophages produce reactive oxygen species during phagocytosis leading to oxidative damage (Carlson *et al.*, 1998). On the other hand Dusart and Schwab argue that inflammatory cells release cytokines and growth factors that may be important for neuroprotection and glial scar formation (Dusart & Schwab, 1994). Chemo-attractant cytokines present due to inflammatory processes also lead to the statement that inflammation may have a beneficial role (Bartholdi & Schwab, 1997;Streit *et al.*, 1998). It has also been shown that an increased number of macrophages following SCI promotes neurite growth therefore promoting neuronal sprouting and regeneration (David *et al.*, 1990;Lotan *et al.*, 1994).

1.2.2 The glial scar

Following SCI, the CNS undergoes reactive gliosis. Glial cells and non CNS cells invade the site of injury to clear up debris and form a wall, known as the glial scar, which prevents secondary pathophysiological damaging mechanisms from spreading (Ramon y Cajal, 1928;Reier PJ *et al.*, 1983;Reier PJ *et al.*, 1986;Fawcett & Asher, 1999). The glial scar acts as a physical barrier and also releases inhibitory chemical substances leading to the prevention of axonal regeneration.

The first components to make up the glial scar are debris from myelin and oligodendrocytes. This is followed by the activation of microglia and the invasion of blood-borne macrophages (Profyris *et al.*, 2004). At this early stage, the glial scars' components, except for the myelin debris is permissive to axonal re-growth (Fawcett & Asher, 1999) After this, the glial scar becomes dominated by components that block axonal growth such as oligodendrocyte precursor cells and meningeal cells which make contact with astrocytes (Fawcett & Asher, 1999). The astrocytes migrate, proliferate, and up-regulate glial fabrillary acidic protein (GFAP) and surround the area of primary lesion and form the bulk of the glial scar (Profyris *et al.*, 2004). Hence, the glial scar with all its inhibitory factors acts as a molecular and physical barrier to axonal growth.

1.2.3 Inhibition in the CNS

Many inhibitory molecules are upregulated in the CNS following SCI. These molecules can be divided into two categories: 1. Myelin associated inhibitory molecules. 2. Molecules synthesised by cellular components of the glial scar which either remain on the surface of these cells or are secreted into the extracellular

matrix (Fawcett & Asher, 1999). During the acute phase of SCI, CNS myelin is the main inhibitor of axonal re-growth. Inhibitory components of myelin include myelin associated glycoprotein (MAG), and oligodendrocyte myelin glycoprotein (OMgP), and NOGO-A.

Inhibitory molecules synthesised by the cellular components of the glial scar can either be solely inhibitory (proteoglycans) or can have both inhibitory and axonal re-growth promoting effects (tenascins, netrins, semaphorins). Chondroitin sulphate proteoglycans (CSPGs) are one of the major inhibitors of the proteoglycans group (Profyris *et al.*, 2004). Members of the CSPGs include NG2, neurocan, versican, brevican, and phosphocan. In addition to acting as inhibitory molecules, tenacins and can also bind to CSPGs retaining them within the vicinity of the glial scar while netrins and semaphorins can also contribute to axon guidance during development (Fawcett & Asher, 1999;Grimpe & Silver, 2002).

1.2.3 Secondary injury

Following SCI, damage in the spinal cord continues, resulting in secondary cell death and consequently, in the damage and death of cells in the tissue surrounding the lesion that was not affected during the early stages of the injury. Shortly after injury, expansion of the haemorrhagic front occurs at the site of trauma (Tator & Fehlings, 1991;Tator, 1995). Next, a penumbra surrounds the primary lesion and oedema occurs mostly in the white matter (Guth *et al.*, 1999). The grey and white matter lose their definition, becoming softer and start to swell due to increased oedema (Tator, 1995). The spinal cord develops cavities, and at later stages these cavities combine to form large cysts, surrounded by glial tissue (Beattie *et al.*, 2002). In some cases, these cysts can extend rostrally and caudally to the primary injury leading to a

condition known as posttraumatic syringomyelia (Tator, 1995). During the more chronic stages of SCI, atrophy of the spinal cord occurs at the region of the primary injury. Spinal cord atrophy can also be seen caudal and rostral to the site of the primary injury due to Wallerian degeneration in ascending and descending tracts (Tator, 1995). Other mechanisms that increase secondary damage include necrosis, infarction, excitotoxicity, and apoptosis.

Therefore, it is clear that a firm understanding of both the inflammatory responses that occur following a SCI and of the mechanisms underlying inhibition and the development of the glial scar is required before intervention and treatment of SCI.

1.3 The rat spinal cord

Before going into the details of the RST and CST, it is vital to know a few important points about the anatomy of the rat spinal cord, and the location of these tracts.

The rat spinal cord consists of 8 cervical segments, 13 thoracic segments and 6 lumbar segments. The cross sectional organisation of the spinal cord is fairly similar along its entire length. The white matter consists of ascending and descending fibres that form the various spinal cord pathways. The grey matter contains cell bodies of neurons. This thesis investigates the RST in control animals and in those with a lesion to the dorsal columns, in which the main part of the CST runs. The location of these two tracts in the spinal cord is illustrated in Figure 1.1.

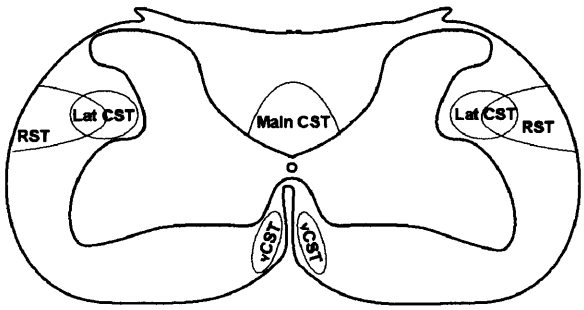


Figure 1.1 Location of the CST and RST in the spinal cord.
Schematic diagram showing the location of the main, crossed CST (main CST), lateral crossed CST (Lat CST), ventral uncrossed (vCST) and the RST in the rat

From dorsal to ventral, the white matter is divided into dorsal, dorsolateral, ventrolateral, and ventral funiculi. The grey matter contains cell bodies of neurons and is divided into the dorsal horn, the intermediate grey, the central grey which surrounds the central canal, and the ventral horn (Figure 1.2).

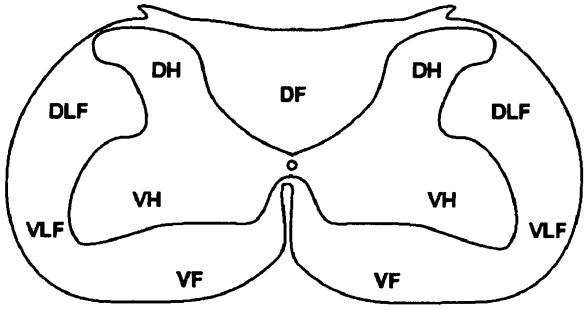


Figure 1.2 Nomenclature of the white and grey matter in the spinal cord.
Schematic diagram of the spinal cord showing the location of the dorsal horn (DH), ventral horn (VH), dorsal funiculus (DF), dorsolateral funiculus (DLF), ventrolateral funiculus (VLF), and ventral funiculus (VF).

The spinal cord is subdivided into 10 dorsoventrally arranged laminae according to Rexed (1952). He divided the laminae on the basis of their cytoarchitectonic characteristics. These divisions are illustrated in Figure 1.3. taken from the rat atlas (Paxinos G & Watson C, 1998).

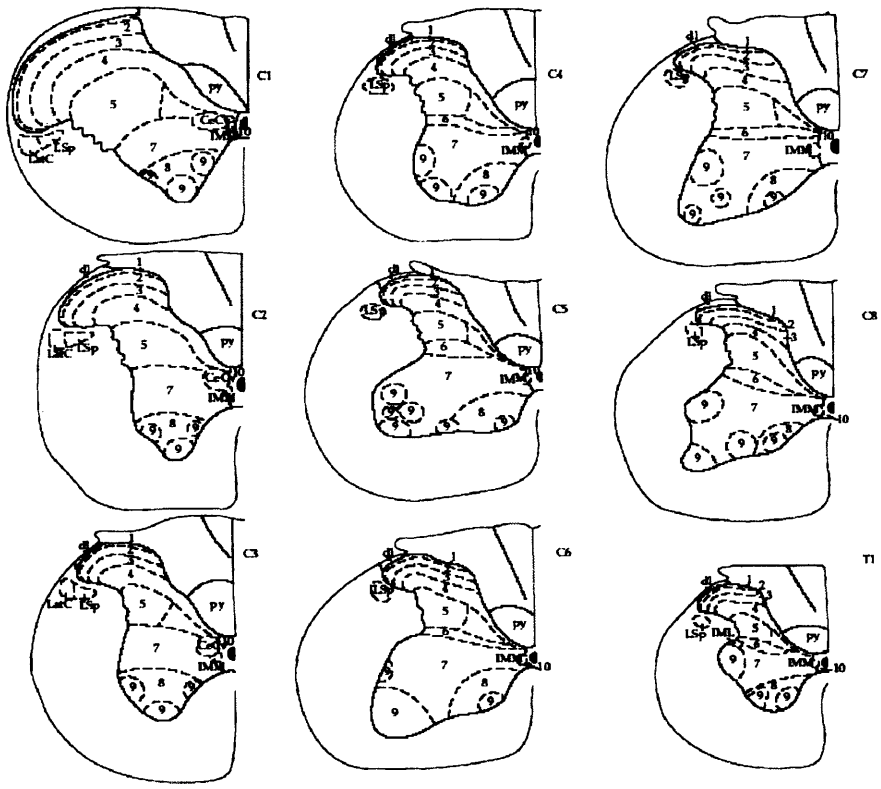


Figure 1.3 Distribution of Rexeds' laminae in the cervical spinal cord of the rat.
Figure taken from the rat atlas (Paxinos G & Watson C, 1998), with permission. CeCV=central cervical nucleus, dl= dorsolateral fasciculus, IML=intermediolateral cell column, IMM=intermediomedial cell column, LatC=lateral cervical nucleus, LSp= lateral spinal nucleus, py= pyramidal tract

1.4 Rat forelimb muscles

Motoneurons that innervate the forelimb muscles are organised in columns and those that innervate flexor muscles are located more medially than those that innervate the extensor muscles in any given spinal segment. The motoneurons innervating the distal musculature of the limb are located caudally in the cervical spinal cord, whilst those innervating the proximal limb are found more rostrally and ventrally (McKenna *et al.*, 2000). It was later shown that motoneurons innervating the proximal (biceps and triceps) part of the limb are distributed in a column ranging from C4-C7. Those

supplying the intermediate part of the forelimb are located in C5-C6 and C7-C8. The motoneurons innervating the forepaw are located more dorsally in the ventral horn of segments C7 and C8 (Kuchler *et al.*, 2002).

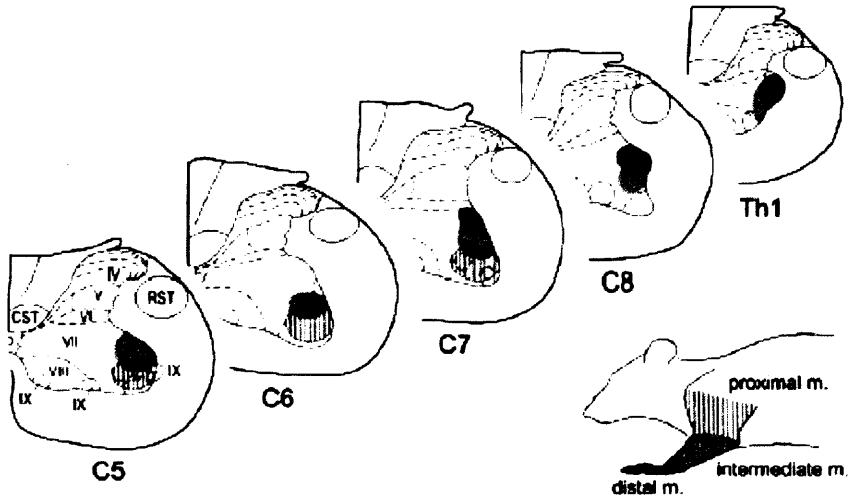


Figure 1.4 Forelimb motoneuronal pools in the rat spinal cord.
Figure demonstrates upper limb motoneuron pools as shown on transverse planes of the spinal cord. Taken from Kuchler et al 2002, with permission.

1.5 The red nucleus and rubrospinal tract

The mammalian red nucleus (RN), which is located in the mesencephalon, has been the subject of numerous morphological studies since the early studies of Mahaim (1894), Von Monakow (1909), and Ramon Cajal (1909-1911). The RN consists of a bilateral aggregation of neurons on either side of the midline and is divided into a larger-celled magnocellular part (RNm, caudal) and a more rostral, smaller-celled parvocellular part (RNp). In the rat, the nucleus extends for a diameter of 1200μm rostrocaudally, 1000μm mediolaterally and 800μm dorsoventrally (Reid *et al.*, 1975). During phylogenesis, the RN changes significantly; in reptiles and birds, the RN is homogenous and contains only large cells whilst in mammals, the RN contains

medium and small sized cells in addition to the large cells. In the rat, the RN neurons can be seen in varying sizes; giant neurons (soma diameter >40μm), large neurons (soma diameter 26-40μm), medium neurons (soma diameter 20-25μm) and small neurons (soma diameter <20μm). The giant and large multipolar neurons are predominantly found in the caudal one-third of the nucleus (Reid *et al.*, 1975).

The rubrospinal tract (RST), which originates from the RN, was first discovered by Von Monakow in 1883 in the dog and since then numerous studies have contributed towards the understanding of physiological and anatomical characteristics of this tract. The origin of the RST was unknown until 1890, when Held, who was working on unborn rats, showed that it came from the contralateral RN.

In the neonatal, developing and mature rat, the RST originates from the caudal 2/3 of the RN, from both the RNm and RNp (Shieh *et al.*, 1983;Canedo, 1997). It decussates at the level of the tegmentum, running in a ventrolateral position through the brain stem and descends along the dorsolateral funiculus (DLF) extending to lumbosacral levels within the spinal cord (Brown, 1974). The rubrospinal fibres projecting to the cervical spinal cord and forelimb motoneurons are located in the dorsal and dorsomedial part of the RN whilst those projecting to lumbosacral levels and hindlimb motoneurons are found in the ventral and ventrolateral portion of the RN (Huisman *et al.*, 1981).

The RST has been identified in many species but there seems to be a lack of evidence for its existence in humans. Von Monakow questioned the existence of the RST in man when he first discovered it, although he later changed his view and stated that the tract did indeed exist in man but was very small. Nathan and Smith also dedicated some studies to the investigation of its existence in man (Nathan &

Smith, 1982). They observed that rubrospinal fibres, immediately after their decussation, run obliquely, posterior to the medial lemniscus till they reach the grey matter anterior to the inferior peduncle. From there, they run caudally entering the lateral part of the lateral lemniscus. Then continue caudally close to the descending tract and nucleus of the trigeminal nerve, entering the medulla anterior to them (Nathan & Smith, 1982). They identified a few large rubrospinal fibres in the upper cervical cord lying immediately anterior to and overlapping corticospinal fibres. Nathan and Smith stated that rubrospinal fibres in man can be identified only during their degeneration as they do not form a compact tract containing only rubrospinal fibres, but instead are intermingled with fibres of other systems. They reviewed evidence for the existence of the RST in man and it was concluded that the large fibres originating from the RNm to make up the RST are small in number and only a few project to the upper cervical segments. However, in humans the RNp is more defined, with a large number of rubro-olivary fibres arising from it to form the central tegmental tract (Nathan & Smith, 1982).

It seems that evidence for the existence of the RST in humans is inconclusive. Most investigators agree that there are only a few large neurons in the RNm and if there is a RST, then it is very small and only extends to the cervical level (see Nathan and Smith 1982 for references).

1.5.1 Projection of the RST and its postsynaptic targets

The RST is one of the most extensively studied tracts in the spinal cord, but there are some contradictory and unresolved issues concerning its course, termination pattern and postsynaptic targets. In the rat, the RST terminates in Rexeds' lamina V and VI, as well as in the dorsal part of lamina VII. Occasionally, RST fibres can be found

projecting to more ventral regions of the spinal grey (Brown, 1974;Kuchler *et al.*, 2002). Whilst the RST is mainly thought to be a contralateral tract, a small number of fibres have been shown to originate from the ipsilateral RN. Following a unilateral injection of horseradish peroxidase into the cervical and lumbar spinal cord of rats, neurons were labelled bilaterally in the RN (Shieh *et al.*, 1983).

Antal and colleagues also showed evidence for an ipsilateral tract that contained 10-28% rubrospinal terminals (Antal *et al.*, 1992). They hypothesised that some of these fibres could represent collaterals of ipsilaterally descending axons, or they could represent axons of those descending contralaterally but that re-cross the midline at the level of the spinal cord. In agreement with this, reports of an uncrossed component have also been described in the rat by other investigators (Shieh *et al.*, 1983;Holstege, 1987;Antal *et al.*, 1992) and contralateral fibres have been demonstrated to re-cross the midline and terminate in the ipsilateral spinal cord (Brown, 1974).

Evidence for an ipsilateral component has also been shown in the cat (Holstege & Kuypers, 1982;Holstege, 1987;Holstege & Tan, 1988)

Some RST fibres have been reported to terminate in lamina IX in the cervical spinal cord of the cat (Holstege, 1987;McCurdy *et al.*, 1987;Holstege & Tan, 1988), rat (Brown, 1974;Kuchler *et al.*, 2002) and monkey (Holstege *et al.*, 1988), although the study by Antal and colleagues found no evidence for fibres terminating on motoneurons (Antal *et al.*, 1992).

In a more recent study, Kuchler *et al* (2002) provided light microscopic evidence in the rat, that boutons from rubrospinal fibres can make close appositions with motoneuron proximal dendrites and cell bodies in the intermediate and distal forelimb

motoneuronal pools. These RST collaterals were frequently seen making appositions with multiple motoneurons (Kuchler *et al.*, 2002). This observation contrasts that of the CST where each main collateral of a cortico-motoneuronal axon establishes very few synaptic contacts (possibly only one) with the dendrites of any one recipient motoneuron as shown in the monkey (Lawrence *et al.*, 1985) and rat (Liang *et al.*, 1991). However, an electron microscopic study provided no evidence for direct cortico-motoneuronal synaptic connections within lamina IX between corticospinal axon boutons and the proximal dendrites of forelimb motoneurons (Yang & Lemon, 2003). In their study, Yang and Lemon found only eight boutons in lamina IX in two rats. Electron microscopic analysis did not provide any evidence for cortico-motoneuronal monosynaptic connections.

In the cat, it was shown that very few contacts are made between neurons in the RN and motoneurons in the spinal cord (Hongo *et al.*, 1969). The effect the RST has on interneurons was investigated and was later shown that volleys in the RST evoke short latency EPSPs in the spinal cord providing evidence for monosynaptic connections between rubrospinal axons and spinal interneurons. Physiological experiments have shown that these neurons may either act as first order interneurons of reflex pathways from group Ib of group II muscle afferents (Hongo *et al.*, 1972) or they may represent last order interneurons projecting directly to motoneurons and receiving monosynaptic or polysynaptic connections from cutaneous afferents. The interneurons represent both excitatory and inhibitory neurons located in lamina V-VII (Baldissera *et al.*, 1971;Hongo *et al.*, 1989a;Hongo *et al.*, 1989b). Antal *et al* (1992) provided direct morphological evidence of this in the rat, where synaptic contacts between rubrospinal terminals and calbindin-D28k (CaBP) reactive dendrites were visualized using electron microscopy (CaBP is a calcium binding protein that is used as a marker for certain subsets of excitatory spinal interneurons). All CaBP containing postsynaptic dendrites were stained

negative for GABA and glycine. These results suggested that rubrospinal terminals establish synaptic connections with excitatory and inhibitory interneurons in the rat spinal cord and showed that a population of the excitatory neurons receiving monosynaptic input are located in lamina V-VI.

Therefore, the signals carried by the rubrospinal axons are conveyed primarily through spinal interneurons to indirectly activate motoneurons controlling limb movement. Inhibitory and excitatory interneurons located in lamina V-VII receive monosynaptic excitation from rubrospinal fibres (Hongo *et al.*, 1972;Baldissera *et al.*, 1981). Stimulation of hindlimb nerves, the RN and the motor cortex while carrying out intracellular recording from interneurons in the cat lumbar cord, has demonstrated convergence of signals from cutaneous afferents, muscle afferents including Ia and Ib, and descending pathways including the CST and RST onto individual spinal interneurons. The method of spatial facilitation was used to test whether two pathways converge on interneurons while recording intracellularly from motoneurons (Illert *et al.*, 1975b;Illert *et al.*, 1976b;Illert *et al.*, 1977;Illert & Tanaka, 1978). This method is based on the principle that two pathways converge if stimulation of one produces a facilitatory or suppressive effect in the other. For example, stimulation in the RN may not produce a response in motoneurons, but when this stimulation is coupled with stimulation in the CST, an EPSP is evoked. Hence, these two pathways must converge somewhere in the spinal cord. Lundberg and colleagues proposed a model that specifies convergence of descending and afferent inputs onto the C3-C4 propriospinal neuron. The propriospinal neuron has a disynaptic linkage with agonist motoneurons and a trisynaptic inhibitory linkage to Ia inhibitory interneurons projecting to antagonist motoneurons (Illert *et al.*, 1975a;Illert *et al.*, 1975b;Illert *et al.*, 1976b;Illert *et al.*, 1977;Illert *et al.*, 1978). This model is illustrated in Figure 1.5.

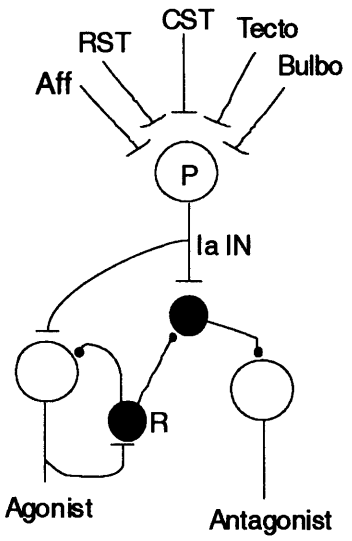


Figure 1.5 Convergence of descending and ascending inputs onto a propriospinal neuron. The C3-C4 propriospinal neurons (P) receive convergent inputs from afferent fibres (Aff), the RST, the CST, the tectospinal tract, and bulbospinal tract. These pathways exert disynaptic excitation of agonist motoneurons through activation of propriospinal neurons. Trisynaptic inhibition to antagonist motoneurons is through Ia inhibitory interneurons (Ia IN). R=Renshaw cell. (Keifer & Houk, 1994), with permission.

1.5.2 Electrophysiological activation of the RST

The RST is one of the major pathways that transmit cerebellofugal influences to the spinal cord in cats. It receives its main excitatory inflow from the interpositus nucleus (Tsukahara *et al.*, 1967).

According to Baldissera *et al* (1972), stimulation of the RN evokes a two component discharge: the direct and synaptic. The direct component had the same latency as the negative antidromic field potential and is thought to be caused by the direct activation of rubrospinal neurons, either by activation of their cell bodies or axons. The second component was thought to be caused by the synaptic activation of the rubrospinal neurons. Both components were abolished following a lesion that interrupted the RST in the medulla. Baldissera *et al* (1972) showed that stimulation ventrally in the RN produced synaptic activation (monosynaptic) of all rubrospinal fibres without any direct activation of them. The shift to direct activation in this region

occurred only when stronger stimulus intensities were applied. Baldissera concluded that stimulation ventral in the RN activates presynaptic fibres, leading to synaptic activation of rubrospinal neurons. This was evidenced by the occurrence of temporal facilitation using weak stimulus strength. Stronger stimulation gave stimulus locked activation indicating a strong synaptic linkage. They hypothesised that these presynaptic fibres activated are the interpositorubral fibres and they confirmed this by recording antidromic responses in the nucleus interpositus. Synaptic stimulation of rubrospinal fibres was less prominent in the dorsal aspect of the RN. The threshold focal point for these two components was located in different areas of the RN. The low threshold focus for the direct activation of the neurons projecting to the lumbosacral cord was found in the hindlimb region of the RN and also in the medial part of the RN where the axons leave. They also found that direct activation can be evoked from a region that extends caudally from the medial part of the RN. The low threshold focus for synaptic stimulation was found at the ventral border of the RN.

1.5.3 Sensory input to the RN

The RST regulates flexor-extensor muscles and consequently regulation of muscle tone. This cannot be done if not correlated to sensory information. This means that sensory information must somehow be passed onto the magnocellular region of the RN. Sensory information may be passed on via afferents which reach the RN via intermediary structures such as the cerebral cortex and the cerebellum. The cerebral cortex and cerebellum receive large afferent inflows from either the spinocerebellar tract or from primary sensory pathways and they have direct connections with the RN (Massion, 1967).

The main afferent pathway to the RN comes from the contralateral interpositus nucleus in the cerebellum and is therefore expected that part of the sensory afferents reach the RN via the cerebellum. Following cerebellectomy in the cat, spontaneous activity in the RN is reduced and remains reduced for several hours indicating that the cerebellum exerts a facilitatory effect on the RN. In chloralose anaesthetised rats, the evoked response to sensory stimulation consisted of an early excitatory phase, a later inhibitory phase and a final prolonged phase of activation. Following a cerebellectomy, rats showed a reduction only in the early response It was concluded that sensory afferents reach the RN via two different routes: a non-cerebellar pathway partly responsible for the first phase and a cerebellar pathway responsible for the prolonged inhibition and delayed activation (Massion, 1967). The pathway that passes through the cerebellum involves a relay in the cerebellar cortical zones which are connected to the contralateral RN through the nucleus interpositus. Direct stimulation of the intermediary zone of the anterior lobe and the paramedian lobule produces inhibition followed by a later excitation in the contralateral RN. This response is identical to the delayed response (inhibition and delayed activation) evoked in the RNm following sensory stimulation. Therefore, the delayed response of the RN is due to reflex activation of the cerebellar cortex by sensory afferents. This is known as the cerebellar reflex arc (Massion, 1967). Stimulation of sensory afferents that pass via the cerebellar reflex arc produces inhibition and excitation in the RN, and produces the opposite effect in postural tone (Massion, 1967). It can therefore be concluded that the cerebellum has two systems that project towards the RN, an inhibitory pathway and an excitatory one (Toyama *et al.*, 1968).

Padel and colleagues argue that there is more a direct somatosensory pathway and provided evidence for this. Electrical stimulation of the paws evokes short-latency EPSPs and IPSPs as recorded from RN cells intracellularly. After decortication and decerebellation, similar responses remained (Jeneskog & Padel, 1984). This

provided evidence for a somatosensory pathway that bypasses the cerebellum and cerebral cortex. Numerous investigations were carried out to identify the ascending pathway responsible for transmitting these signals. It was later found that low intensity stimulation of the dorsal columns induced large EPSPs and IPSPs in RN cells. Stimulation of the medial lemniscus (ML) also elicited monosynaptic EPSPs in the same cells. From these studies it was concluded that the peripheral signals reached the RN via the dorsal columns and the ML after a relay in the dorsal column nuclei (Padel & Jeneskog, 1981;Jeneskog & Padel, 1984;Robinson *et al.*, 1987). However, they also discussed the possibility of the RN pathway ascending from the ventral spinal cord. To address this, they lesioned the dorsal columns in the most rostral segments of the spinal cord of decorticate and decerebellate cats and observed that the somatosensory responses in the RN remained (Padel *et al.*, 1986). From this, they concluded that the pathway to the RN which relays at segmental levels the signals from primary afferents running in the dorsal columns and ascending in the ventral part of the spinal cord. This pathway was identified as a spinorubral pathway which was later shown to originate from spinothalamic fibres giving of collaterals at the midbrain level (Relova & Padel, 1989). The question still remained as to whether the rubral responses are transmitted via the dorsal column-medial lemniscal pathway, with a relay in the dorsal column nuclei or through the ascending pathway from the ventral spinal cord, or even both. Lesions were carried out on the frontal cortex, the cerebellum, and the dorsal columns at C2-C3. Recordings were then made intracellularly from RN cells, and also from the dorsal root entry zone at C4-C5, while stimulating the dorsal columns either rostral (C2), or caudal (C4) to the lesion. Stimulation of forelimb and hindlimb peripheral nerves was also carried out. Stimulation of the dorsal columns, both caudally and rostrally evoked large EPSPs and IPSPs in RN cells. Stimulation of the peripheral nerves gave excitatory and inhibitory responses in RN cells (Rathelot & Padel, 1997). Next, in addition to the above lesions, a dorsal hemisection was carried out. Stimulation of

the dorsal columns rostral to the lesion did not produce a post-synaptic potential, but stimulation caudally did. They concluded that the signals from the dorsal columns to the RN are relayed at segmental levels (Rathelot & Padel, 1997). As responses were seen rostrally and caudally to a dorsal column lesion, they could not attribute the rostral responses solely to the spinorubral pathway as dorsal column nuclei and segmental relay cells coexist rostrally to the lesion at C2-C3. To clarify this, in non decerebellate and decorticate cats, the dorsal columns were lesioned on one side at C4-C5, dissected free rostrally over two segments, and lifted and placed on two hook stimulating electrodes. Stimulation to this dissected part produced no responses in the RN. Dorsal column stimulation at C2 evoked a large EPSP-IPSP pattern in the RN. This implied that the dorsal columns must be left in place for signal transduction to the RN. The dissection of the dorsal columns disrupted the connections between dorsal column fibres and cells located at segmental levels relaying signals to the RN. Thus, this implies that the dorsal column-medial lemniscal pathway, with a synaptic relay in the dorsal column nuclei does not induce a response in the RN (Rathelot & Padel, 1997). Hence, the experiments of Rathelot and Padel (1997) conclude that, in the cat, with the cerebellum and frontal cortex lesioned, the somatosensory signals to the RN are transmitted via the spinorubral pathway running in the ventral part of the spinal cord, and relaying at segmental levels.

Stimulating both the ML and the ventral funiculus of the spinal cord produced monosynaptic responses in RN cells, which were similar in shape suggesting that they were generated by the same ascending fibres. They confirmed this by running a series of double shock tests, and concluded that some fibres ascending in the ventral part of the spinal cord project monosynaptically to rubrospinal cells (Rathelot & Padel, 1997).

The RN therefore receives sensory input both directly and indirectly. The indirect inputs are relayed through the cerebellum and sensorimotor cortex and the direct inputs originate in the spinorubral pathway.

1.5.4 Function of the RST

Electrophysiological studies

The RN has been implicated in the control of different muscles of the forelimbs and hindlimbs. In 1957, Pompeiano showed that the RST exerts facilitatory influences on flexor alpha-motoneurons and inhibitory ones on extensors (Hongo *et al.*, 1969). However, Hongo and colleagues showed that there are also excitatory effects on extensor motoneurones and inhibitory effect on flexors (Hongo *et al.*, 1969). Destruction of the RN in the cat showed very little if any paresis of the limbs, leaving the animal capable of righting, standing and walking. However, other motor abnormalities were observed such as a mild increase in extensor tonus, deficiencies in placing and hopping reactions and slight ataxia (Hongo *et al.*, 1969).

It was later shown that the projection of the RST is greater in segments that control distal muscles than that of proximal muscles in the cat (Holstege, 1987;McCurdy *et al.*, 1987;Holstege & Tan, 1988) and the monkey (Humphrey *et al.*, 1984;Holstege *et al.*, 1988). This anatomical data was supported by electrophysiological data that showed in the awake monkey, that the RNm activity is highly modulated during movements involving distal joints of the forelimb and hindlimb (Cheney, 1980;Mewes & Cheney, 1991). In addition to this, it was demonstrated in the monkey that many cells in the RNm are more strongly related to movements involving the distal joints than proximal joints (Houk *et al.*, 1988). To strengthen these findings, intracellular

recordings in the cat following stimulation in the RNm produced EPSPs preferentially in motoneurons of distal muscles rather than proximal muscles (Fujito *et al.*, 1991). However, recent studies have demonstrated that RNm activity is more strongly modulated during multi-joint reaching movements than in single joint movements (Mewes & Cheney, 1994). This could suggest that the RNm may be more involved in coordinating distal and proximal joints together instead of movements involving only distal or proximal joints. Belhaj-Saif *et al* (1998) later confirmed, in the macaque monkey, that the RNm controls both proximal and distal muscles of the forelimb, although influences over the distal muscles are predominant. They also showed that the RNm output preferentially excites extensor muscles at both the distal and proximal level and that the majority of sites within the RNm co-facilitate muscles at both proximal and distal joints. This would suggest that the RNm may be preferentially involved in the control of movements requiring coordination of proximal and distal joints.

The RN is also thought to indirectly control motor actions by conveying signals from the motor cortex to the spinal cord (Massion, 1967). In 1902, Probst showed that after the sectioning of the pyramidal tract, stimulation of the motor cortex still produced movement in the contralateral muscles of the cat (Massion, 1967). These movements disappeared after sectioning of both the RST and the pyramidal tract. Von Economo and Karplus believed that the corticorubral pathway supplements the action of the pyramidal tract (Massion, 1967). A direct connection between the RNm and the pyramidal cortex forms the basis of this corticorubral pathway which is somatotopically organised and allows a localised action to be exerted through rubrospinal fibres onto contralateral flexor muscles. Tsukahara and Kosaka showed that stimulation of the motor cortex in the cat excited neurons in the RNm of the ipsilateral RN, most probably in a monosynaptic manner and also confirmed that these cortico-rubral connections are excitatory. It is therefore concluded that the

cortical control of the RNm is carried out through a monosynaptic pathway that originates in the pyramidal cortex. Many studies have also confirmed that the CST can be functionally replaced by the corticorubral pathway. For example, Evans and Ingram (1939) demonstrated in the cat, that when the CST or the RST was lesioned, the remaining intact tract compensates for the loss of the other. Similar results were obtained by Goldberger in the macaque (Goldberger, 1965).

Behavioural studies

The RST has been shown to be implicated in precise limb movements, a function that parallels that of the CST. Although lesions in the RN of the rat produce subtle deficits during reaching, a behavioural task that requires precise limb positioning and fine digit control, this deficit is much more significant when combined with a lesion of the CST (Whishaw *et al.*, 1990;Whishaw *et al.*, 1998). In the rat, the activity of RN neurons during a forelimb grasping task shows a very close similarity to that of the CST with regard to the distribution of onsets of neuronal spikes. It was shown that the distribution of changes in neuronal activity (excitation and inhibition) relative to the phase of reach to grasp movements are similar in the RN to those reported for the motor cortex in the rat (Hyland, 1998;Jarratt & Hyland, 1999). These electrophysiological findings strengthen the role of the RST in hand movement control. In a study that compared the effect of lesions to the dorsal columns, the DLF, and the ventrolateral funiculus at the cervical level of a rat spinal cord, the most severe impairment was seen following the DLF lesion (Schrimsher & Reier, 1993). Reduced digit flexion and grasping ability was noted. In the monkey, lesions to the RST, either at the spinal cord or brain stem level, resulted in impaired movement of the distal forelimb and hand, and a loss of digit flexion, which was more prominent following the DLF lesion (Lawrence & Kuypers, 1968b). The DLF contains other

pathways in addition to the RST, and the combined effect of damage to these pathways may cause a more severe deficit in digit flexion, explaining the slight difference in deficits in RN lesions versus DLF lesions. However, Muir and Whishaw (2000) reported that lesions to the RN do not seem to produce major deficits in precise hand movement but did induce permanent locomtor gait deficits. But, when the RN lesion is combined with a lesion of the motor cortex, these deficits become more significant (Whishaw *et al.*, 1990).

This evidence may lead to the conclusion that the RST is involved in a more general control of forelimb muscle groups whilst the CST controls the individual distal limb movements. Activity from the RN was recorded during various forelimb movements in the rat (Jarratt & Hyland, 1999) and the monkey (Gibson *et al.*, 1985;Houk *et al.*, 1988) and showed that the RN is more involved in giving a timing signal for coordination of movements across the joints.

Although it seems that the RST and CST are important in target reaching and food taking, there is evidence suggesting that the C3-C4 propriospinal system can mediate control of both reaching and food taking movements in the cat. See next section.

1.6 The C3-C4 propriospinal system

The RST and CST appear to be important in target reaching and food taking and there is evidence that points towards the control of these tasks via the C3-C4 propriospinal system. This section will include a brief overview of the C3-C4 propriospinal system of the cat, rat, monkey, and also of humans.

In limb segments, propriospinal neurons were defined as those that originate outside the segments to a limb and project mainly into them (Illert *et al.*, 1977). In 1941 Lloyd first hypothesized that effects from higher centres including those from the motor cortex are relayed through propriospinal neurons. This hypothesis was later investigated by Stewart and colleagues by producing lesions in the CST at various segmental levels. They discovered that excitation of hindlimb motoneurons was transmitted via propriospinal neurons originating between C4 and L3 (Stewart *et al.*, 1968).

The C3-C4 propriospinal system was later disclosed by investigating its synaptic actions on forelimb motoneurons (Illert *et al.*, 1976a), where it was demonstrated that volleys in corticospinal fibres evoked disynaptic EPSPs in forelimb motoneurons. Illert and colleagues then performed a study to disclose the location of this system (Illert *et al.*, 1977). Lesions of the DLF were performed affecting the RST and CST at different levels rostral to forelimb motoneurons. Transmission to forelimb motoneurons was interrupted when the CST and RST were transected at C2 or rostral C3 but not when transected at C5. This therefore provided evidence that the propriospinal neurons originated in the C3-C4 spinal segments and ran in the ventrolateral funiculus. Illert and colleagues also demonstrated that the axons of the C3-C4 propriospinal neurons are located in the ventral part of the lateral funiculus, as evidenced by a lesion in this area that abolished the EPSPs mediated by their axons (Illert *et al.*, 1977). Monosynaptic convergence was demonstrated on propriospinal neurons coming from the pyramidal and rubral pathway as well as tectospinal fibres and primary forelimb afferents. This convergence was disclosed indirectly by the facilitation of pyramidal disynaptic EPSPs and revealed monsynaptic facilitation from cutaneous and group I muscle afferents (Illert *et al.*, 1977).

Because the propriospinal axons are located ventrally in the spinal cord, it is possible to lesion the RST and CST which are located dorsally, without affecting the propriospinal neurons. Alstermark and colleagues showed that following a dorsal C5 lesion which eliminates the input to forelimb segments whilst maintaining the input to the C3-C4 propriospinal neurons, leaving the propriospinal system intact, cats performed normal target reaching but were unable to perform a food taking task; which means that they were unable to grasp and supinate the forepaw to retrieve the food which was located in a tube. However, following a dorsal C2 lesion (C3-C4 propriospinal system lesioned) there was severe impairment in target reaching, in which the cat was not even able to lift its paw. Therefore, one can conclude that the C3-C4 propriospinal neurons can mediate the command for target reaching, but the command for food taking depends on a group of interneurons lying in the same segments as the motoneurons innervating the forelimb muscles. With a C5 ventral lesion transecting the C3-C4 propriospinal axons, but leaving the CST and RST intact the deficits seen were extremely mild where the food taking task remained normal, while the target reaching task became slightly ataxic. A C2 ventral lesion had no effect whatsoever on both target reaching and food taking (Alstermark *et al.*, 1981b). Therefore, although the command for target reaching can be mediated by spinal interneurons in the forelimb segments, this can not be carried out with the same effectiveness as by the C3-C4 propriospinal neurons. It therefore seems that transmission to forelimb motoneurons is interrupted when transected at C2 but not when transected at C5. These results lead to the belief that these lesions cause an interruption of the cortico and/or rubrospinal input to the C3/C4 propriospinal neurons (Alstermark *et al.*, 1981a;Alstermark *et al.*, 1981b).

As previously mentioned, complete transection of the RST and CST at C5 in the cat causes complete loss of food taking. However, there was a slow recovery of function over several months, which was attributed to takeover by the reticulospinal tract

(Alstermark *et al.*, 1981b). Following a complete transection of the CST in the cat with little or no damage of the RST, food taking was not affected. When a second lesion was carried out destroying the RST, food taking was again not affected leading to conclude that there is a rubrospinal then a reticulospinal takeover of commands in this serial lesion experiment. However, the ability to carry out the food taking task was lost when a C2 ventral lesion was carried out (Alstermark *et al.*, 1987). Therefore, it seems that the C3-C4 propriospinal neuronal command can be mediated by cortico, rubro, or reticulospinal pathways under certain conditions. However, a recent study provided evidence that the C3-C4 propriospinal system has the ability to mediate control of both reaching and food taking movements in cats (Blagoveshchenskii *et al.*, 2005). Table 1.1 summarises the affects of the various lesions:

	C3/C4	RST	CST	Target reaching	Food taking
Dorsal C5	✓	×	×	✓	×
Dorsal C2	✓	×	×	Severe impairment	
Ventral C5	×	✓	✓	✓	Ataxic
Ventral C2	×	✓	✓	✓	✓

Table 1.1 Effects of various lesions on target reaching and food taking.
The first columns states the four lesions carried out, the next three columns state whether these lesions destroyed the C3/C4 propriospinal neurons (column 2), the RST (column 3), or the CST (column 4). The last two columns indicate whether target reaching or food taking was affected. ✓ not affected by lesion, × affected by lesion.

Although a previous study has provided evidence in favour of the existence of monosynaptic corticomotoneuronal connections in the rat (Liang *et al.*, 1991), experiments by Alstermark and colleagues strongly suggest that these connections do not exist (Alstermark *et al.*, 2004). They carried out a series of lesions in the spinal

cord in order to selectively interrupt the transmission of specific descending pathways. Transection of the CST in the dorsal columns at C2 or C5 or both (sparing the resticulospinal tract) abolished the direct corticospinal volley and the negative cord dorsum potentials. However, with 2 or 3 shocks, a synaptic volley was seen to arrive in C6-C7. Alstermark suggested that the fastest pyramidal EPSPs are disynaptically mediated by reticulospinal neurons with their axons running in the ventral half of the spinal cord and project monosynaptically to forelimb motoneurons. They also stated that the EPSPs cannot be mediated by vestibulospinal or tectospinal pathways as they do not receive direct input from the CST and the RST was also excluded because it had been lesioned. Corticospinal disynaptic EPSPs were abolished when a C5 dorsal column lesion (transecting the CST) was added to a previous C2 hemisection (corticobulbospinal system transected, C3-C4 propriospinal system spared). Alstermark suggested that these results provided evidence that the C3-C4 propriospinal neurons do not mediate disynaptic corticospinal excitation to forelimb motoneurons and that the disynaptic excitatory corticoreticulospinal pathway could be viewed as functionally analogous to the C3-C4 propriospinal system in the cat (Alstermark *et al.*, 2004).

Transecting the corticoreticulospsinal pathway and propriospinal axons at C5 (sparing input to forelimb segments via segmental interneurons), did not abolish the pyramidal EPSPs. This suggested disynaptic and trisynaptic excitation in forelimb motoneurons can be mediated by segmental interneurons in C6-C8.

In conclusion, the results Alstermark showed suggests a weak disynaptic excitatory linkage together with a stronger trisynaptic or polysynaptic pathway.

Data presented by Nielsen and colleagues at the IUPS meeting (2001, abstract 2245) also argued against the existence of monosynaptic corticospinal projections to

cervical motoneurons of the rat. Intracellular recording from cervical motoneurons evoked EPSPs with latencies longer than 2ms, which were recruited mainly with three shocks. Another population of neurons showed latencies of 1.2 and 1.8ms and were recruited with one shock but were greatly facilitated with three shocks. The IPSPs generally had a latency of about 1-2ms longer than the EPSPs. Nielsen therefore suggested that the most direct pathway is most likely to be disynaptic, possibly mediated by the C3-C4 propriospinal neurons but the majority of motoneurons received excitation through a polysynaptic pathway. This data was published recently (Nielsen *et al.*, 2007).

It has been demonstrated, as in the rat, that the mouse also lacks monosynaptic corticomotoneuronal excitation but in contrast, the mouse had weak corticospinal excitation to forelimb motoneurons (Alstermark & Ogawa, 2004). This was explained as being due to the more medial termination of corticospinal fibres in the grey matter than that in the rat. This pattern of termination avoids the lateral part of lamina VII where many last order interneurons are located. They also found that pyramidal excitation could be mediated via a fast disynaptic cortico-reticulospinal pathway.

Further experiments carried out in the monkey showed a lack of evidence for the existence of significant corticospinal excitation of motoneurons via C3-C4 propriospinal neurons. Lemon and colleagues were unable to find evidence for significant di- or oligosynaptic excitatory projections to upper limb motoneurons in the macaque monkey when stimulating corticospinal axons in the pyramidal tract (Maier *et al.*, 1998;Alstermark *et al.*, 1999). A similar result was also found in experiments with the squirrel monkey, a lower primate where hand dexterity is less advanced than that in the macaque. The results obtained in the squirrel monkey were intermediate between the macaque and the cat, where evidence for such connections was demonstrated but they were weaker than that of the cat (Nakajima *et al.*, 2000).

Alstermark and colleagues repeated the experiments in the macaque with the additional step of administering strychnine to suppress postsynaptic inhibition (Alstermark *et al.*, 1999) and managed to demonstrate weak di- and oligosynaptic corticospinal EPSPs. Alstermark suggested that the weakness in transmission was due to inhibition of the propriospinal neurons, whilst others suggested that this weakness was due to a coticomotoneuronal takeover where the coticomotoneuronal system effectively replaces propriospinal mediated control (Maier *et al.*, 1998;Nakajima *et al.*, 2000). However, Alstermark and colleagues argued that difficulties encountered by Maier *et al* (1998) and Nakajima *et al* (2000) to activate the C3-C4 propriospinal system in the macaque monkey was due to a stronger feed-forward pyramidal inhibition in the propriospinal neurons compared with the cat (Isa *et al.*, 2006;Alstermark *et al.*, 2007).

Since 1988, Pierott-Deseilligny and colleagues have carried out experiments to demonstrate whether this system existed in humans. Reflex studies in man supported the existence of a C3-C4 propriospinal system that is under powerful inhibitory control (Pierrot-Deseilligny, 1996) and was demonstrated that in humans, facilitation of propriospinal neurons from peripheral and corticospinal sources can be reversed to inhibition if the strength of either input is increased suggesting that there is a strong inhibitory control of transmission through the presumed propriospinal system (Nicolas *et al.*, 2001). These findings indeed do suggest that the propriospinal system does exist in humans. Although it was argued that if a propriospinal system does exist in humans, it would be of little functional importance due to the evolutionary changes in the propriospinal system (Lemon, 1999), while others state that these changes are more involved with the extent to which propriospinal neurons are subject to feed-forward and feedback inhibition rather than to its actual existence (Burke, 2001).

1.7 Regeneration and plasticity following spinal cord injury

It has been known since the time of Ramon y Cajal that effective regeneration of lesioned axons does not occur in the CNS (Ramon y Cajal, 1928). This failure is normally attributed to the formation of scar tissue and accumulation of inhibitory molecules round the lesion site. In 1911 the first successful experiment aimed at regeneration demonstrated that differentiated CNS neurons can regenerate (Massion, 1967). In this study peripheral nerve pieces were transplanted into the cortex of young rabbits and silver staining techniques showed bundles of fibres entering the graft and growing along the denervated Schwann cell bands. This study received a lot of criticism and the results were neglected until the 1980s when Aguayo and colleagues used nerve grafts to test the regenerative capacity of spinal cord, brain and optic nerves (David & Aguayo, 1985;Friedman & Aguayo, 1985;Keirstead *et al.*, 1985;Munz *et al.*, 1985;So & Aguayo, 1985). The success of Aguayo's experiments was attributed to the fact that inhibitory substances present in the CNS are absent in the PNS and also because peripheral nerve grafts contain cells that secrete neurotrophins which help promote axonal growth.

Foetal spinal cord transplants provide a trophic environment for axotomised neurons. It has been demonstrated that transplants of foetal spinal cord rescues immature axotomised rubrospinal neurons from retrograde cell death in newborn rats with a mid-thoracic lesion (Bregman & Reier, 1986). Horseradish peroxidase tracing showed labelled neurons within the transplant and RN neurons contralateral to the spinal cord lesion. Foetal spinal cord transplants when combined with neurotrophic factors, which are key nervous system regulatory proteins that modulate neuronal survival, axonal growth, synaptic plasticity and neurotransmission, provide an environment that helps in promoting regeneration in the spinal cord. In a study using adult rats, brain derived neurotrophic factor (BDNF) secreting fibroblasts applied

acutely to the cervical spinal cord at the injury site of the adult rats promoted regeneration of rubrospinal axons through the fibroblast graft and into the distal spinal cord (Liu *et al.*, 1999). The RST axons grew for long distances (4-5 segments caudal to the graft) within the spinal white matter and terminated in the grey matter of the spinal cord. This regeneration was accompanied with behavioural functional recovery that showed significant recovery of forelimb usage. However, when fibroblasts were implanted 5 and 6 weeks after injury, the regeneration of rubrospinal axons was less than that demonstrated in the acute model (Jin *et al.*, 2000). It therefore seems that such a lengthy delay in BDNF application is not as affective as a more acute application. This was confirmed in a study where the delayed application of BDNF (2 months after injury) at the lesion site of adult rats was shown to be unable to rescue chronically axotomised rubrospinal neurons (Kwon *et al.*, 2004).

In addition to promoting regeneration, the administration of BDNF reduces loss of rubrospinal neurons either via atrophy (severe shrinkage) or retrograde cell death in the adult rat (Liu *et al.*, 2002). The use of neurotrophins in preventing neuronal cell death was also demonstrated in a study where BDNF, neurotrophin-3 (NT3), and nerve growth factor (NGF) was administered exogenously (acutely) following a spinal cord hemisection in the newborn rat (Diener & Bregman, 1994). Here, axotomised RN neurons were rescued from retrograde cell death and this effect was maintained in the presence of BDNF but only transiently with NT-3 and NGF. BDNF was also shown to prevent rubrospinal neurons from atrophy when applied to their cell bodies acutely, and increases their expression of Growth Associated protein-43 (GAP-43) and Tα1 tubulin (Kobayashi *et al.*, 1997;Kwon *et al.*, 2002). GAP-43 is a marker for differentiating neurons, where GAP-43 expression increases in developing and regenerating neurons and Tα1 tubulin is usually expressed at high levels when

neurons extend processes. In another study it was shown that when a 2 week delayed transplant of foetal spinal cord with neurotrophins was applied to a transected spinal cord of an adult rat, axonal regeneration and recovery of function was higher than that when applied acutely. Retrograde labelling showed regeneration of rubrospinal neurons under these conditions following a complete transection at the mid-thoracic level (Coumans *et al.*, 2001).

A more recent study made use of an adeno-virus vector model that mediated gene transfer of BDNF also demonstrated the regenerative capacity of rubrospinal neurons in the rat spinal cord. Following a complete transection at T8, the vectors were injected into both rostral and caudal stumps of the lesioned spinal cord. BDA was used as an anterograde tracer whilst fluoro-Gold was used as a retrograde tracer to assess the regeneration of the rubrospinal fibres. Fluoro-Gold labelled neurons were found in the RN and BDA labelled fibres were found distal to the transection (Koda *et al.*, 2004). Other studies utilised ciliary neurotrophic factor (CNTF) which was shown to rescue some rubrospinal neurons from cell death when applied to the lesion site 4 and 8 weeks after injury, but this was not observed when the application was delayed for 14 and 22 weeks (Houle & Ye, 1999). In addition to viral vectors, adult human olfactory neuroepithelial derived neurosphere-forming cells (NSFCs) produce BDNF and are used as a tool to administer BDNF. NSFCs grafted into the cervical spinal cord of the adult rat one week after a partial unilateral C3-C4 cervical hemisection survive, migrate, and integrate into the host spinal cord (Xiao *et al.*, 2005). It was shown that the NSFCs rescued axotomised RN neurons from atrophy and promoted axonal regeneration accompanied by functional recovery. Retrograde tracing with flurogold revealed that RST axons regenerated 4-5 segments caudal to the graft and anterograde tracing with BDA confirmed regeneration of the RST axons through the transplant and into the white matter 3-6 segments caudal to the graft. This study also visualised a few axons that terminated in grey matter close to motoneurons. In a later

study, RST regeneration was assessed fourteen weeks post-lesion and it was shown that RST fibres regenerated and were seen in the white matter 4-8 segments caudal to the graft (Xiao *et al.*, 2007). It was also demonstrated that RST fibres re-established synaptic connections with ventral horn motoneurons of the distal cervical spinal cord.

There are therefore numerous cellular and molecular strategies that could be used to help promote regeneration in the spinal cord (Thuret *et al.*, 2006;Bradbury & McMahon, 2006b). These strategies quite often involve transplanted cells in combination with neurotrophic factors. The following lists a few examples, and select references. Transplanted cells include Schwann cells (Keyvan-Fouladi *et al.*, 2005;Fouad *et al.*, 2005), foetal cells (Bregman & Reier, 1986;Bregman *et al.*, 2002), olfactory ensheathing cells (Li *et al.*, 1997;Keyvan-Fouladi *et al.*, 2003), ,and stem cells (Lu *et al.*, 2003). Another important strategy is to neutralize myelin inhibitors such as NOGO, oligodendocyte myelin glycoprotein (Caroni *et al.*, 1988), and chondroitin sulphate (Barritt *et al.*, 2006b).

However, as discussed above, the CNS has limited capabilities to repair following a spinal cord injury (SCI), and successful therapeutic interventions aimed at coaxing lesioned fibres to regenerate are limited. However, it is known that the CNS is capable of extensive reorganisation following an injury to the spinal cord, which can lead to a certain level of functional recovery. This is especially true following an incomplete SCI. During the chronic phase of human SCI, it has been known that patients show some sensory and/or motor recovery, which most probably occurs without CNS regeneration or reinervation (De Leon *et al.*, 1998a;De Leon *et al.*, 1998b). Plasticity can either occur as an effect on the synaptic transmission, by modifying synaptic strength (synaptic plasticity), or anatomical plasticity can occur whereby new circuits form through sprouting and anatomical reorganisation.

Understanding the mechanisms that underlie plasticity may allow the development of new treatments for SCI. In animal models, several studies have demonstrated that spontaneous functional recovery can be a result of plasticity.

Various studies of SCI in animal models have suggested that motor recovery results from the development of compensatory movements (Alstermark *et al.*, 1987;McKenna & Whishaw, 1999) or through the plastic properties of the CNS (Raineteau & Schwab, 2001). It has been estimated that if as little as 10% of descending spinal tracts are spared, some voluntary control of locomotion can be recovered (Basso, 2000). In a recent study, evidence was provided for spontaneous functional recovery to be a result of synaptic plasticity within the spinal cord (Gulino *et al.*, 2007).

It has long been hypothesized that the magnocellular region of the RN compensates for those motor impairments that are caused by lesion of the pyramidal tract and reorganization of rubrospinal output could contribute towards functional recovery. Following a unilateral CST lesion in the monkey, partial recovery of foot and arm control occurred. However, when the RN was lesioned, this recovery disappeared (Lawrence & Kuypers, 1968a;Lawrence & Kuypers, 1968b). This could be due to the takeover of function by the RN, which is lost once the RN is lesioned.

Alstermark *et al* (1981) observed that if as little as 20% of the RST was spared following a lesion to the DLF at C4/C5, recovery of food taking in the cat is much faster and more complete. When a lesion to the DLF was carried out sparing most of the RST, food taking was unaffected (Alstermark *et al.*, 1987). One month later, the DLF was completely transected at C5/C6 and the food taking behaviour was still present. It was suggested that the remaining RST fibres could induce reticulospinal pathways to take over the command for food taking (Alstermark *et al.*, 1987). More

recently, also in the cat, it was shown that a small percentage of RST fibres (4-6%) may enhance the speed of recovery (Pettersson *et al.*, 2000). It was therefore suggested that the small number of RST fibres spared are unlikely to alone mediate the command for food taking, but instead they function to facilitate recovery via ventrally located pathways (reticulospinal). Pettersson hypothesised that the mechanism underlying this takeover is that based on associative plasticity. By this, it is meant that when the command for food taking is passed from the remaining rubrospinal fibres to interneurons which also receive input from reticulospinal fibres, the interneurons are depolarised facilitating the induction of a plastic enhancement in transmission in reticulospinal synapses (Pettersson *et al.*, 2000).

Recently, it was shown that the RN-mediated output on forearm spinal motoneurons reorganise in a profound way (Belhaj-Saif & Cheney, 2000). In their study, Belhaj-Saif and Cheney assessed whether the RNm contributed to compensation for motor impairments associated with lesions of the pyramidal tract in the rhesus monkey. For this they used stimulus-triggered averaging of electromyographic activity from forearm muscles to characterize changes in motor output from the RN following lesions to the pyramidal tract. Their results demonstrated a clear pattern of post-lesion organization of RN mediated output effects on forearm muscles. Five years before recording, a unilateral pyramidotomy was carried out destroying 65% of the left pyramidal tract. The normal prominent extensor preference in excitatory output from the RNm was reduced in the lesioned monkey. Suppression effects which are normally more prominent in flexor than in extensor muscles were more evenly distributed after recovery from the pyramidal lesion. A change in the organization of the RNm following a pyramidal tract lesion therefore seems to promote recovery of forelimb motor function.

Various studies have provided evidence for anatomical plasticity in the RST. Raineteau and colleagues carried out a number of studies on a rat model of SCI. In this model, the CST was completely transected and the animals were treated with an antibody (mAb IN-1) that neutralizes the neurite growth inhibitory protein Nogo-A. Anatomical studies of the RST showed that the number of collaterals innervating the cervical spinal cord doubled in the mAb In-1 treated rats. They also showed that low threshold microstimulation of the motor cortex induced a rapid forelimb electromyography response that was mediated by the RN in the mAb IN-1 treated animals but not in the controls (Raineteau *et al.*, 2001;Raineteau *et al.*, 2002).

Plasticity can also be enhanced by targeting CNS growth inhibitory components, which can be divided into two main categories. The first one comprises constituents of myelin such as NOGO-A, myelin associated glycoprotein (MAG), and oligodendrocyte myelin glycoprotein (OMgp, for a review on myelin inhibitors, see (Filbin, 2003). Nogo-A is produced when the Nogo gene is differentially spliced into three isoforms: A, B, and C. Nogo-A is the longest variant and contains a unique amino terminal sequence (amino-Nogo). The carboxyl terminal of Nogo-A is common to all three isoforms and contains two trans-membrane domains separated by a short extra-membrane region (Nogo-66). Both Nogo-66 and amino-Nogo are inhibitory. Nogo-66 signals through Nogo-66 receptor (NgR) and p75 neurotrophin receptor (p75NTR) to activate RhoA and downstream pathways, causing growth cone collapse. Myelin associated glycoprotein (MAG) and oligodendrocyte myelin glycoprotein (OMgp) also exert their effects via this pathway. Work by Strittmatter and colleagues demonstrated that following a spinal cord lesion and blockade of NgR, sprouting of corticospinal and raphespinal fibres can be seen (Li & Strittmatter, 2003;Li et al., 2005)

Treatment with antibodies raised against Nogo-A result in the neutralisation of Nogo and can promote regeneration in the CNS. For example, treatment with mAb IN-1 following a lesion to the CST at the level of the pyramids in the rat promoted sprouting of fibres across the midline into the contralateral pons and the formation of new synapses (Blochlinger et al., 2001). It was also shown that following a unilateral lesion of the motor cortex and mAb IN-1 treatment, fibres of the intact CST sprouted into the contralateral denervated RN and pons (Wenk et al., 1999).

The second category of components is that of the extracellular matrix molecules such as chondroitin sulphate proteoglycans (CSPGs). CSPGs are important components of the glial scar and perineuronal nets. The enzyme chondroitinase ABC (ChABC) can reduce the inhibition by removing the glycosaminoglycan side chains. It has recently been demonstrated that following a lesion to the dorsal columns and treatment with ChABC, sprouting of both injured corticospinal fibres and intact serotonergic fibres and also intact primary afferents can occur (Barritt *et al.*, 2006a). In another recent study, ChABC was injected into the cuneate nucleus following a lesion to the ipsilateral dorsal columns at C6-C7. Electrophysiological receptive field mapping of the cuneate nucleus demonstrated expansion of receptive fields and anatomical evaluations demonstrated sprouting of intact afferents (Massey et al., 2006).

From the above studies, it is clear that targeting CNS inhibitors are vital in promoting axonal growth following spinal cord injuries (for reviews see (Bradbury & McMahon, 2006a;Priestley, 2007).

However, recovery following spinal cord injury is often seen without any pharmaceutical or surgical intervention. It is known that following complete spinal transections, cats slowly recover locomotion on a treadmill. This was first described

by (Sherrington, 1910;Rossignol, 2006) published an article reviewing plasticity and its effect following spinal cord lesions. This is based on the concept of central pattern generation in which rhythmic activity is produced in neurons in the absence of sensory input which was first demonstrated by Grillner & Zangger (1975 and 1979)(Grillner & Zangger, 1975;Grillner & Zangger, 1979)(Grillner & Zangger, 1975;Grillner & Zangger, 1979)(Grillner & Zangger, 1975;Grillner & Zangger, 1979)(Grillner & Zangger, 1975;Grillner & Zangger, 1979)(Grillner & Zangger, 1975;Grillner & Zangger, 1979). For such locomotion to be achieved, significant plasticity probably occurs in the pathways that control locomotor function. In humans with a clinically complete SCI, it has been shown that a locomotor pattern can be generated (Dietz, 1995;Dietz et al., 1995) and it is known that exercise can facilitate the recovery of locomotion following a SCI (Edgerton & Roy, 2002;Edgerton et al., 2004). Recent work by Edgerton and colleagues has shown that exercise increases the expression of BDNF and NT3 in the spinal cord (Gomez-Pinilla et al., 2002;Ying et al., 2003) and that BDNF plays a role in synaptic plasticity (Ying et al., 2005). Locomotor training has also been shown to benefit patients with SCI (Edgerton *et al.*, 2001;Wirz *et al.*, 2005).

Activity dependent plasticity can also be induced via conditioning of the H reflex, an electrical analogue of the spinal stretch reflex, which is evoked by direct stimulation of the primary afferent resulting in an improved motor function (Chen *et al.*, 2006;Lavrov *et al.*, 2006;Wolpaw, 2007).

Plasticity therefore plays an important role in the recovery of function following a SCI.

1.8 The Corticospinal tract of the rat

Within this thesis, a particular set of experiments is involved in the destruction of the main corticospinal tract (CST) in order to assess whether there are any physiological and/or anatomical changes in the RST and whether this affects the behavioural outcomes of the rats as assessed using specific behavioural tests. This subsection gives a brief introduction into the CST of the rat, its origins, projections and function.

In mammals, the termination pattern of the CST overlaps extensively with that of the RST, and it is thought that the CST may compensate for damage to the RST following an injury to the spinal cord (see below).

1.8.1 The origin of the CST and its projection pattern

The CST is a long descending pathway and is found only in mammals. It arises from the Betz neurons in layer V of the somatosensory cortex in the cat (Chambers & Liu, 1957) and monkey (Kuypers, 1960), and from the sensorimotor cortex in the rat (Brown, 1974;Miller, 1987)and opossum (Martin & Fisher, 1968). Its axons pass through the internal capsule and cerebral peduncle where they course ventrally in the brainstem and decussate at the level of the medulla (Terashima, 1995). Corticospinal fibres descend along the spinal cord reaching lumbosacral segments and project mainly to the dorsal horn and intermediate grey of the spinal cord (Brown, 1971;Terashima, 1995).The CST also extends to lamina I and II in the cervical and lumbar enlargements of cats and monkeys (Cheema *et al.*, 1984) and also in rats (Casale *et al.*, 1988).

In the rat, the CST can be divided into a crossed and an uncrossed component. The main crossed CST descends in the ventral part of dorsal columns (Brown, 1971). A small number of CST fibres have been reported to be seen in the ipsilateral dorsomedial, contralateral lateral, and ipsilateral ventromedial funiculi of the rat spinal cord (Casale *et al.*, 1988;Rouiller *et al.*, 1991;Brosamle & Schwab, 1997). According to Terashima, the ventral uncrossed component makes up 1-3% of the whole CST (Terashima, 1995). Corticospinal fibres have been shown to send collaterals to various brain stem nuclei including the RN, pontine nuclei, inferior olivary nuclei, and dorsal column nuclei. (O'Leary & Terashima, 1988). It has been demonstrated that corticospinal fibres innervate neurons in lamina IV to lamina VI (Casale *et al.*, 1988) and have been shown to make some close appositions to motoneurons in the cervical cord (Liang *et al.*, 1991).

1.8.2 Function of the CST

The CST has an important role in motor function, in reaching and grasping behaviour. A pyramidotomy resulted in deficits in grasping and digit use in the rat (Schrimsher & Reier, 1993;McKenna & Whishaw, 1999). It has been shown that recovery in pellet retrieval following a dorsal CST injury (dorsal columns) is once again lost following a ventral CST injury (Weidner *et al.*, 2001). It is therefore hypothesised that the ventral CST compensates for the loss of the main CST. It has also been proposed that the CST can compensate for damage to the rubrospinal tract via activation of the rubro-olivary projection (Kennedy, 1990). It was shown that transection of the rubrospinal tract before lesioning the RN led to facilitation of recovery of motor activity (Kennedy & Humphrey, 1987;Fanardjian *et al.*, 1999). A similar situation occurs when the sensorimotor cortex is lesioned prior to transection

of the bulbar pyramid (Fanardzhyan *et al.*, 2002). This recovery in activity is not present when the early lesion to the sensorimotor cortex or the RN is absent.

1.8.3 The CST and its similarity to the RST

As for its similarity to the RST, axons of both the lateral corticospinal and rubrospinal systems travel in the lateral funiculus of the spinal cord and influence both distal and proximal muscles of the forelimb (Belhaj-Saif *et al.*, 1998). It has been shown that rubrospinal fibres terminate in the same region of the spinal cord as the corticospinal fibres as seen in the monkey (Lawrence & Kuypers, 1965) and cat (Pompeiano & Brodal, 1957) In these species, fibres from both the RST and CST descend in the DLF of the spinal cord. However, in the rat and opossum, the CST descends in the most ventral part of the dorsal funiculus (Martin & Fisher, 1968). In the rat, It extends to lumbosacral levels and projects to the dorsal horn (Brown, 1971). It has been suggested that the CST in the rat may be more concerned with the modulation of incoming afferent information than with motor control (Brown, 1974). There is evidence that the RN receives afferents from the same region of the sensorimotor cortex which gives origin to the CST and it has been suggested that where the CST is impaired, the RST is capable of motor control and will compensate for the damage in the CST (Massion, 1967). Both the CST and RST are preferentially involved in the control of distal muscles (Lawrence & Kuypers, 1968a;Lawrence & Kuypers, 1968b) and contribute to the initiation of movement.

1.9 Rationale of thesis

The current thesis is aimed at developing an animal model aimed at assessing plasticity and regeneration in experimental models of spinal cord injury (SCI). Following a SCI, and during the chronic stages, spontaneous functional recovery often occurs. However, it is often not known what mechanisms are involved in the recovery. The RST and CST are widely used in experimental models of SCI due to their anatomical and functional organisation. Information from these two tracts converges onto common interneurons representing integration of synaptic signals. Both these tracts are involved in the control of distal muscles and contribute to the initiation and execution of movement. It has also been reported that the RST can compensate for damage to the CST and anatomical reorganisation of this tract can occur following injury to the CST. Here, these two tracts are used to assess possible plasticity in the spinal cord following a SCI. The tract to be lesioned is the CST and assessments are carried out to the RST which include electrophysiological, behavioural, and anatomical assessments. Little work has been carried on the physiology of the terminal organisation of the RST in the rat. This information is required to help understand the mechanisms of recovery. For example, although regeneration or plasticity may be defined anatomically, information is still required on the physiological connections made following an injury. In the current thesis, the electrophysiological organisation of the RST is investigated in the caudal cervical spinal cord of control animals and in those with a lesion to the dorsal columns (C5). To assess spontaneous functional recovery, three behavioural tasks were implemented, the pellet retrieval test, the cylinder test, and the sticker removal test. Anatomical tracing of the RST was carried out in control animals and in those with a dorsal column lesion.

Chapter 2. Electrophysiological actions of the RST

2.1 Introduction

Following spinal cord injury, spontaneous functional recovery can occur. This recovery can be the result of regeneration or reorganisation of pathways and it is often unknown which mechanisms result in the recovery. Pathways within the central nervous system often reorganise and produce new circuits following an injury to the spinal cord. This could occur to both spared pathways and to those damaged following an injury. This reorganisation is defined as plasticity. If recovery does occur, many questions must be answered:

1. Is the recovery a result of plasticity or regeneration
2. If its plasticity, does this plasticity occur in spared pathways or in damaged pathways?
3. Following regeneration or plasticity, do the fibres make functional connections?

Electrophysiological studies are required to answer these questions. Here, the electrophysiological distribution of one pathway has been assessed following a lesion to another. For this, the rubrospinal tract (RST) was chosen due to its well defined functional and anatomical characterisation. The RST originates from the red nucleus, and here this nucleus was selectively stimulated and the actions of the RST in the cervical spinal cord were assessed. Once the distribution of the electrophysiological actions of the RST was characterised, the distribution of its actions were once again assessed in animals with a lesion to the corticospinal track and any changes in its synaptic actions were elucidated.

The results of these experiments will be correlated to those obtained in the anatomical and behavioural experiments.

2.2 Methods

Experiments were carried out on female Sprague Dawley rats weighing between 200 and 290g. Prior to the experiment, the electrode used for recording and stimulating was calibrated for position, as was a rigid sharply pointed stainless steel rod (spike) mounted in an electrode carrier. For this, the alignment of the electrode at 90° was first checked to confirm that the tip was straight. The ear bars of the stereotaxic frame were arranged so that the distance between their tips was 0.2mm. The angle of the electrode was set at 15° to the sagittal plane and the electrode was centred between the ear bars. The anteroposterior (AP), the left-right (LR), and the height (H) values were noted. The spike was calibrated in the same way.

2.2.1 Anaesthesia

During this study, a number of anaesthetic protocols were used in order to determine the best anaesthetic, i.e. for which the condition of the animal was most stable. In the early part of this study, anaesthesia was induced with a solution of ketamine and xylazine (100mg/kg + 5mg/kg respectively, I.P). During the later stages of this study, and in most experiments, animals were anaesthetised with urethane (Sigma, 1.4 g/kg, 30% in saline I.P), and were maintained under the same anaesthetic. Adequacy of anaesthesia was assessed by the absence of palpebral reflexes, limb withdrawal, and changes in blood pressure following a noxious pinch to the hind or forepaw. Under neuromuscular blockade, adequacy of anaesthesia was assessed by

observing the changes in blood pressure following a noxious pinch to the hind or forepaw.

2.2.2 Surgery

Animals were placed on a heat blanket (Harvard homoeothermic blanket control unit) and rectal temperature was maintained between 37º C and 39º C. During some periods, a heating lamp was also used. Prior to surgery, animals were given solumedrone (25 mg/kg, I.M) in 2 doses once at the beginning, and once before the laminectomy to prevent brain and spinal cord oedema and atropine sulphate was administered to prevent bronchial secretions (120μg made up to 1ml saline, I.P). A midline incision was made in the neck, and using blunt dissection the right jugular vein was exposed. Branches leaving the vein were cauterised and an intravenous cannula was inserted. The right carotid artery was exposed and the vagus nerve was separated from the artery with a glass rod and the artery was cannulated. The jugular vein was used for the administration of fluids and anaesthetic. The artery was connected to a pressure transducer for monitoring of blood pressure. While exposing the artery, the trachea was also exposed and it too was cannulated in order to allow artificial ventilation and the monitoring of expiratory CO_2. Animals received a glucose solution (1g in 20ml Hartmann's solution, I.V, Baxter health care LTD as required). Blood pressure was kept within physiological limits by infusion of fluids and any blood lost was substituted with gelofusine, a plasma substitute.

Once canulations were complete, a midline incision was made from the nape of the neck to the thoracic area, as determined by feeling for the T2 dorsal process. The underlying muscles were dissected and retracted to expose the vertebrae from C1 to T2. The laminae from C1-T2 spinal segments were removed to expose the spinal cord. The animal was transferred to a stereotaxic frame (David Kopf instruments,

California, USA) and the head was fixed in the horizontal plane as defined by the rat atlas (Paxinos G & Watson C, 1998). The spike was placed at calibrated coordinates on the skull surface and their coordinates checked against the atlas. A small craniotomy was made in the right parietal bone (centred at 2.6mm lateral to the midline and 2.7mm rostral to the interaural line). The dura was opened with a 27g needle and fine forceps. The spinal cord was stabilised by clamping the dorsal spinal process of T2. A longitudinal incision of the dura-mater exposed the spinal cord, which was then covered with mineral oil. For the intraspinal recordings, small patches of the pia-mater were removed in the selected segment with fine forceps and a 27 gauge needle. All recordings were made under artificial ventilation and with neuromuscular blockade (Pancuronium bromide 2mg/ml initial dose 0.4ml then 0.27ml/hr, I.V).

2.2.3 Recording and stimulating

The experimental layout is illustrated in figure 2.1. A monopolar glass coated tungsten micro-electrode (Merrill & Ainsworth, 1972), was located in the midbrain in the region of the red nucleus (RN), AP approximately 2.7 rostral to the interaural line, 15º to the vertical and at the same LR coordinates noted in the calibration procedure. The atlas showed that at AP 2.7 rostral and at 15º to the vertical, an electrode will pass through the centre of the RN. Cord dorsum potentials were recorded with a silver ball electrode coupled to conventional differential amplifiers (CDP; negativity upwards; reference electrode placed in muscles). All signals were digitally recorded (15 KHz), averaged (at least 16 sweeps) and analysed using Signal (Cambridge Electronic Design). Bipolar stimulation (0.2 msec) of the contralateral dorsolateral funiculus (DLF) at the cervical enlargement was produced with a pair of fine electrodes located on the surface of the DLF and separated by about 1 mm rostro-

caudally. A volley was recorded from the ipsilateral cord dorsum of C2 or C3. Simultaneous recordings were made from the contralateral RN. Tracking was made in and around the red nucleus and a final location of the microelectrode was selected, usually where the antidromic responses were largest. Once the final location of the electrode was determined, the same tungsten electrode was used to stimulate the RN using monophasic cathodic pulses (0.2 msec; 0.7 – 1.8 Hz, stimulating current was monitored in the ground return). On some occasions, short trains of stimuli of up to 750 Hz were delivered. Depending on the experiment, other sites near the red nucleus were stimulated. Orthodromic volleys were recorded in the CDP from various sites on the cord dorsum of a chosen cervical segment (C6 or C7), and also along several segments of the contralateral and sometimes ipsilateral cervical spinal cord for calculation of conduction velocities. When recording along the cervical cord, the distance between each recording was noted. At the end of all experiments, a micro-lesion was produced in the midbrain (approx 10 μA; up to 120 sec) for histological confirmation of the final stimulation site.

Intraspinal field potentials (FPs) were recorded with carbon fibre micro-electrodes (0.4-0.8 MΩ, Carbostar, Kation Scientific) and a IA Axoprobe amplifier (Axon Instruments). FPs were recorded (C5-C8) from a transverse grid of sites separated in depth by 100μm from the surface of the spinal cord to a depth of 2.0mm and usually separated by 100μm in the medio-lateral direction. At the end of the recording, marking electrodes were inserted to define two reference tracks and were left in place.

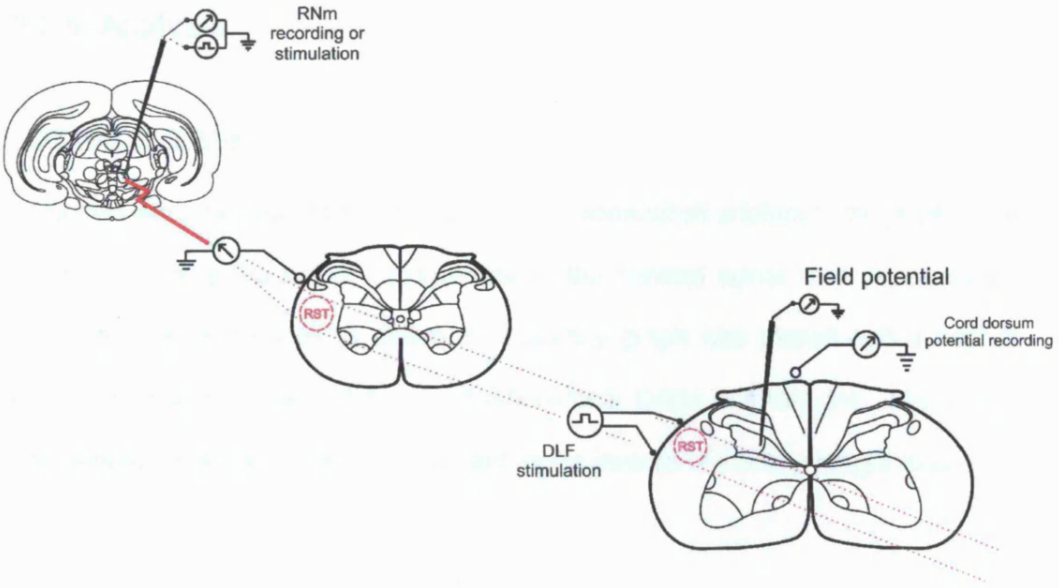


Figure 2.1 Schematic of methods.
Using stereotaxic coordinates, a tungsten electrode was inserted into the red nucleus. Its location was confirmed by the recording of antidromic field potentials while stimulating the contralateral dorsolateral funiculus in the cervical enlargement. Once the location was verified, the same electrode was used for stimulation and recordings were made from the dorsum of the cervical spinal cord. With the use of a carbostar electrode, intraspinal field potential recordings were made (C6-C8) and an isopotential map was created.

2.2.4 Acute DLF lesion

During three terminal experiments, the DLF was lesioned in order to confirm the origin of the synaptic potentials seen in the caudal cervical spinal cord while stimulating the contralateral RN. For this, once the final location of the electrode in the red nucleus was confirmed, the region of maximum synaptic potentials in the contralateral spinal cord (C7) was determined. The DLF of C3 ipsilateral to the recorded fields was then severed with fine forceps. Measurements of CDPs above and below the lesion together with intraspinal recordings from below the lesion were repeated.

2.2.5 Analysis

Conduction velocity

Latencies were measured from the onset of the stimulation artefact to the peak of the positive and negative waves recorded along the cervical spinal cord as measured from the averaged traces. A distance to latency graph was plotted and a best fit linear regression line was determined (Micrococal Origin software Inc., MA, USA). The conduction velocities were calculated as the inverse of the linear regression.

Isopotential maps

The distribution of the electrophysiological actions of the RST in a selected segment of the spinal cord was obtained from plots of isopotentials derived from the FPs. Offline; the potentials from each site were averaged after removing traces with obvious artefacts (e.g. ECG waveforms) or large unit spikes. From each averaged record, the amplitude of the potential was measured at fixed latencies. These latencies corresponded to the peak negativity either for the descending volley, or for the synaptic potential. The isopotential plots were obtained using interpolation algorithms in MatLab (MathWorks) and were then superimposed onto the histologically reconstructed spinal cord section. Using these figures as references, the isopotential plots were then superimposed onto representative spinal cord section taken from the Paxinos' rat atlas.

2.2.6 Histological processing

At the end of all experiments, the animal was perfused intracardially with a solution of heparinised saline followed by a 4% formaldehyde solution (VWR International Ltd, UK) in 0.2M PBS with 5% sucrose. The brain and spinal cord were recovered and

post-fixed at 4°C with the same solution. The spinal cord and brain were blocked. For the spinal cord, the segment corresponding to that where synaptic potentials were recorded was identified by the location of the marking electrodes. The side contralateral to the marking electrodes was marked. The brain was placed in a brain blocker and the area of the red nucleus was identified by reference to the rat atlas (Paxinos G & Watson C, 1998) and the block was cut and extracted from the blocker. The side contralateral to the RN used for stimulation and recording was marked. Both the brain and spinal cord blocks were then cryoprotected with a solution of 30% sucrose in a 4% formaldehyde solution for at least three days. Cross sections (frozen, 50μm) of the brain and spinal cord were produced (cut rostral to caudal) using a freezing microtome (JUNG SM 1400, Leica), and were mounted on gelatine-subbed glass slides. The slides were dried, stained with cresyl-violet acetate (Sigma-Aldrich Company Ltd), cover-slipped with a DePex mounting medium (VWR International Ltd, UK) and allowed to dry. The stimulation site of the red nucleus and electrode tracks of the recorded segment of the spinal cord were visualised using a Zeiss Axioskop microscope (Zeiss, W. Germany). The electrode tracks and spinal cords were reconstructed using the microscope and a drawing tube. Photomicrographs were taken of the stimulation site.

2.2.7 Dorsal column lesion

Animals were anaesthetised with halothane (5% for induction, maintained at 1.8-2.2%, O_2 0.8L/min) and placed on a heating blanket. The temperature was monitored with a rectal probe. The region of the back corresponding to the cervical spinal cord was shaved and cleaned with a Betadine solution. A midline incision was made and the overlying muscle was dissected and retracted to expose the vertebrae. The lamina of fourth spinal segment was removed and bleeding was prevented by

electro-coagulation. The dura was opened using a 27g needle and fine scissors. The dorsal columns including the CST were lesioned with fine scissors and dura-film was placed over the affected spinal cord. The wound was close in layers. The animals were given 5ml sterile saline S.C, 0.03ml Duphamox I.M, and 0.015ml vetergesic I.M. The animal was placed under a heat lamp for recovery.

2.2.8 Assessment of lesion extent

Following perfusion, and post fixation, the spinal cord was extracted and cryoprotected in a solution of 30% sucrose in formalin. The spinal segments correlating to the lesion were identified and removed from the remaining spinal cord. The block was then cut (50μm) using a freezing microtome and mounted onto gelatine coated glass slides and allowed to dry. Slides were stained with a solution of cresyl violet acetate (Sigma-Aldrich Company Ltd) and cover slipped using a DePex mounting medium (VWR international Ltd, UK).

The lesion was visualised using a light microscope. To reconstruct the lesion, the outline of the spinal cord was first drawn under dark field using a drawing tube. The extent of the lesion was then reconstructed by drawing the lesion outline from a number of adjacent sections which correlated to those sections with lesion pathology. The criteria were loss of tissue in the white and grey matter, as well as the appearance of gliosis and cysts. Dark field was also used to assess the absence of white matter tracts. Using a light box, the reconstructed drawings were superimposed onto each other to produce the final lesion reconstruction.

Figures 2.1a was chosen to illustrate an animal with a lesion that did not cause damage to the DLF which was classified as one with a specific lesion to the dorsal columns (R606). The other animal (R575, Figure 2.1b) illustrates a lesion that extends to the DLF, although still only partial. In figure 2.1a it is clearly evident that

the lesion does not extend to the DLF, as the dorsal horn is still intact, although gliotic (arrow, Figure 2.1a, z). In figure 2.1b, the formation of a cyst is illustrated (Figure 2.1b, x) and damage has extended to the left dorsal horn. It is clear that lesion pathology has extended to the DLF, mostly on the left.

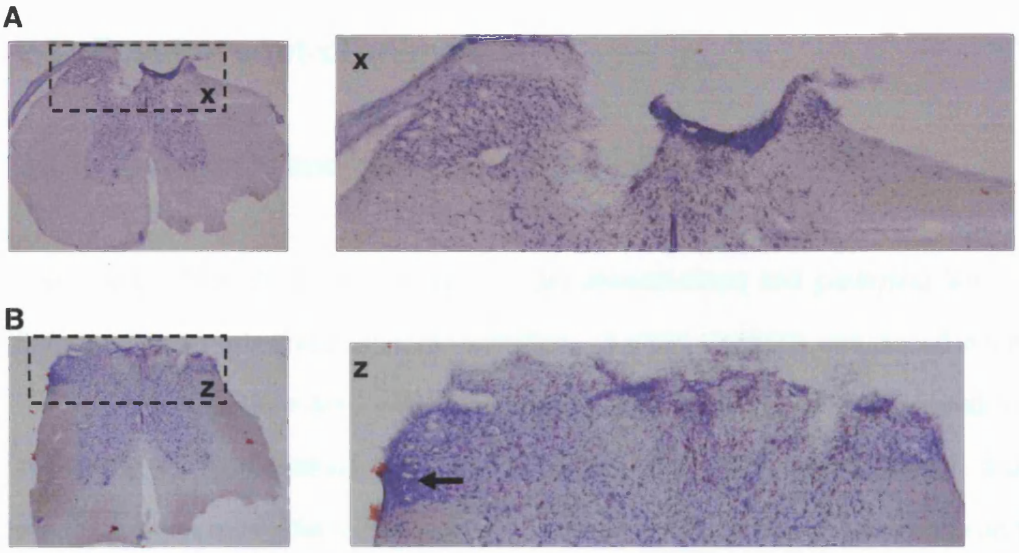
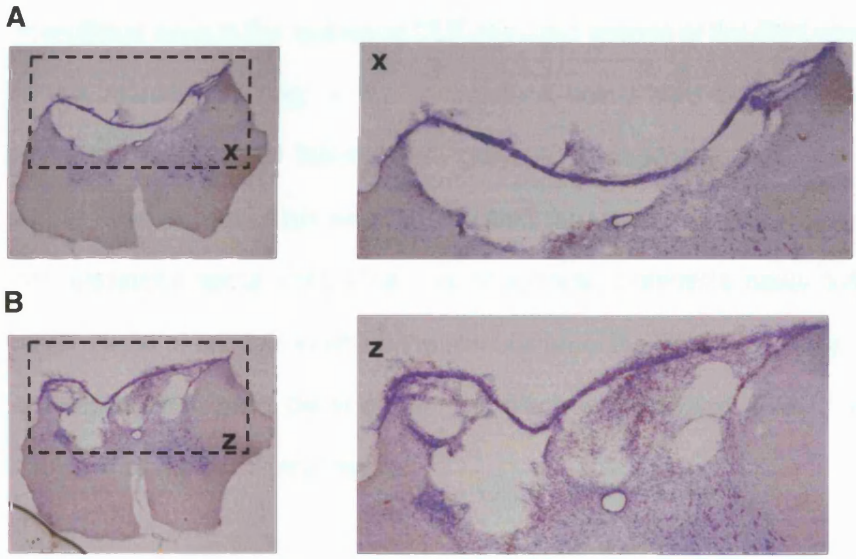


Figure 2.1a Lesion extent of an animal with a dorsal column lesion (R606)
A Cresyl violet stained section illustrating lesion extent at the border of the lesion area, **x** magnification of boxed area in A illustrating the "normal" dorsal horn on the left, **B** Section shows the focus of the lesion, **z** magnification of boxed area in B illustrating gliosis in the dorsal horn (arrow) and dorsal columns hardly extending into the DLF, **C** Reconstruction of lesion, shaded area illustrates area of damage



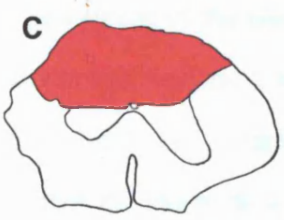


Figure 2.1b **Extension of dorsal column lesion to the DLF (R575)**
A Cresyl violet stained section illustrating lesion extent,
x magnification of boxed area in A shows the formation of a cyst in the dorsal columns, note lesion pathology clearly extends to the left DLF, **B** Section shows the focus of the lesion, **z** magnification of boxed area in B illustrating the cyst and gliosis in the dorsal horn and dorsal columns, **C** Reconstruction of lesion, shaded area illustrates area of damage

2.3 Results- control animals

2.3.1 Antidromic and cord dorsum potentials

The results of this study were obtained from anaesthetised and paralysed female Sprague Dawley rats under artificial ventilation. A micro-electrode was placed in the RN by combining stereotaxis with the recording of antidromic potentials elicited by stimulating the contralateral DLF (Hongo *et al.*, 1969). Stimulation through this electrode in the red nucleus (RN) produced distinct potentials in both the contra- and ipsilateral cervical spinal cord. These potentials depended on the location of the electrode within the midbrain. Intraspinal recordings were made in the caudal cervical spinal cord and maps of field potential recordings were produced from which the actions of the RST were inferred. In general, an early and/or a late descending volley were seen in the contralateral dorsolateral funiculus (DLF) and an early volley was sometimes seen in the ipsilateral DLF. Synaptic actions of the RST were seen mainly in the intermediate grey of the contralateral spinal cord but were also sometimes seen in the ipsilateral intermediate grey. An exception to this was animal R478, where synaptic potentials were seen within the ventral quadrant of both the contra- and ipsilateral spinal cord. The foci of synaptic potentials never extended to the motor nuclei of animals in which the stimulation to the RN was specific. These results are described in more detail below. The electrophysiological actions of the RST are shown in seven intraspinal maps.

The location of the electrode in the red nucleus was confirmed by the recording of antidromic field potentials elicited by stimulation of the contralateral dorsolateral funiculus in the caudal segments of the cervical spinal cord. In order to avoid the midline the electrode was inserted into the midbrain at an angle of 15º. At this angle,

the target depth from the surface of the brain to the red nucleus as calculated from Paxinos' atlas is 7.2mm. However, in our animals, the RN was found more dorsally (usually at a depth of 6.4-6.8mm). Following stimulation to the DLF of the caudal cervical spinal cord, a volley was seen in the CDP recording from the ipsilateral spinal cord in the more rostral segments and a more complex response was seen in or near the contralateral RN. Figure 2.2 illustrates a track through the RN in which an antidromic response in the RN started to appear at a depth of 5.8mm reaching maximum amplitude at a depth of 6.4mm. The latency of the first positive response usually occurred at 1.21 ± 0.16ms (n=21) while the latency to the peak negativity was 1.76 ± 0.3ms (n=23). The antidromic responses usually consisted of a positivity followed by a sharp negativity probably caused by the activation of a population of red nucleus neurons, and was sometimes followed by the activation of one or two single units.

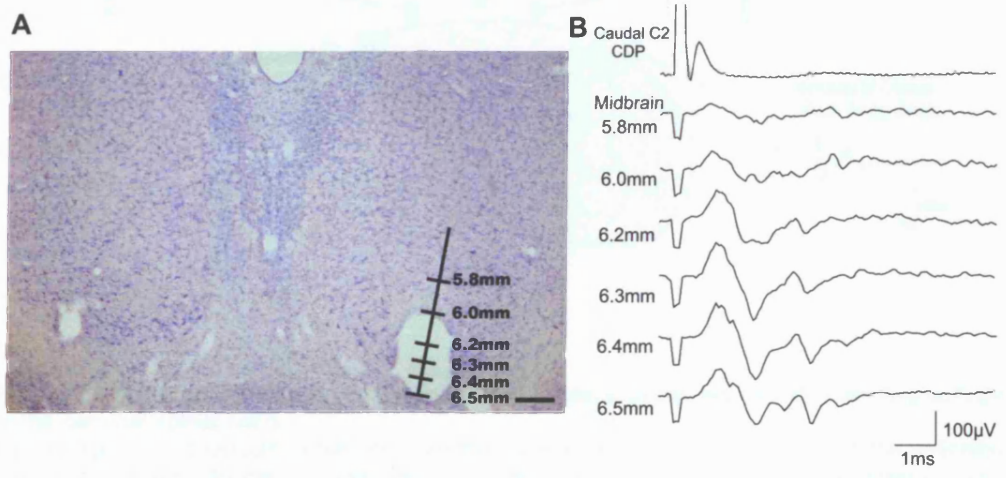


Figure 2.2 Antidromic action potentials in the RNm.
Following stimulation of the contralateral DLF in caudal segments of the cervical spinal cord, an antidromic action potential was seen in the red nucleus. A Photomicrograph of red nucleus and recording track. The hole in the tissue on the right is the marking lesion made at the end of the experiment on this track.Scale bar 200μm. B Antidromic action potentials recorded at the indicated depths. In this and subsequent figures the CDPs are recorded –ve up and midbrain recordings are recorded +ve up and recordings show the average of 16-64 sweeps.

Once the location of the electrode in the red nucleus was confirmed by the recording of antidromic field potentials, the same electrode was used to stimulate the RN. Stimulation of the red nucleus most often produced two descending volleys in the cervical spinal cord, as recorded from the cord dorsum. A late volley (latency to maximum negativity about 1.8ms at C7) appeared only in the contralateral spinal cord while an early volley (latency to maximum negativity about 1ms at C7) appeared in the ipsilateral and sometimes the contralateral spinal cord (figure 2.3). The threshold ranged from 10-80μA for the early volley and from 2-50μA for the late volley.

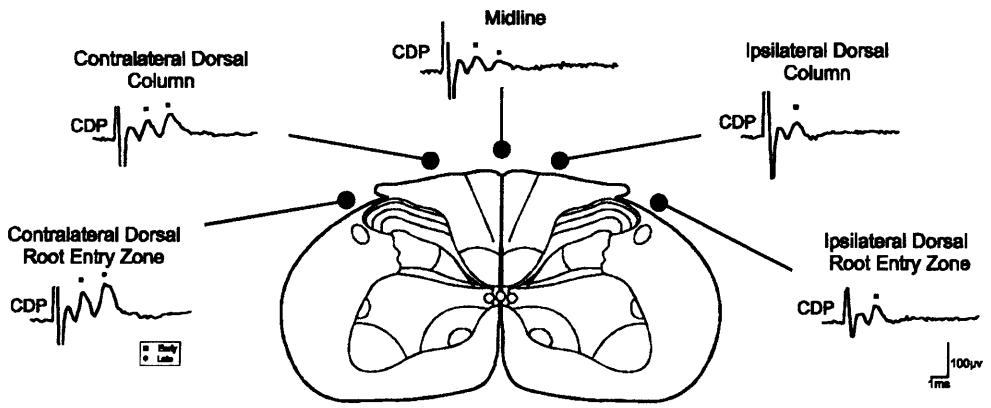


Figure 2.3 A Single stimulation pulse to the red nucleus produced two descending volleys in the cervical spinal cord.
Recordings of cord dorsum potentials from the surface of the spinal cord (C6) at the indicated funiculi are shown. An early descending volley (■) could be recorded bilaterally, while the late volley (●) was only seen in the contralateral spinal cord. All traces were obtained with a single pulse (70 μA, 200 μs).

Data taken from a number of experiments are illustrated in table 1 which shows the stimulation site and the corresponding potentials produced in the cord dorsum of the contralateral and ipsilateral spinal cord. All animals were under urethane anaesthesia except for R410. The stimulation site in the midbrain is indicated in the

photomicrographs and the distance from the caudal edge of the RN is calculated from the number of sections. The caudal edge of the RN was chosen to calculate as a reference for two reasons: 1. At the caudal end the RN neurons are larger and can be seen clearly. This is less clear at the rostral end of the RN as the RN neurons are much smaller and it is difficult to define the most rostral end of the RN. 2. The caudal part of the RN is where the stimulation electrode was targeted as the RST originates from the caudal 2/3 of the RN (Shieh *et al.*, 1983). In order to compare the volleys seen in the contra- and ipsilateral spinal cord, examples were chosen from each experiment where the contra- and ipsilateral CDPs were recorded from the same segment of the spinal cord while stimulating in the RN with the same stimulus strength. The experiments are ordered in the table according to the location of the stimulation site, starting with the most caudal stimulation sites and ending with the more rostral ones. The most caudal stimulation site was seen in animals R474 and R533. The most rostral stimulation site was found in animals R521, where the electrode track was seen 950μm rostral to the caudal edge of the RN. It is important to note here that the RN extends 1200μm rostrocaudally, 1000μm mediolaterally, and 800μm dorsoventrally (Reid *et al.*, 1975).The dimensions of the RN are similar in the animals used in this study. From the data presented in table 1, it can be seen that the presence of these potentials depended on the location of the stimulation electrode in the midbrain.

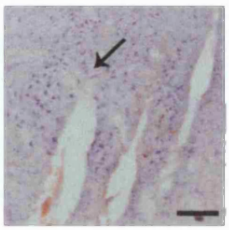
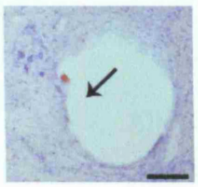
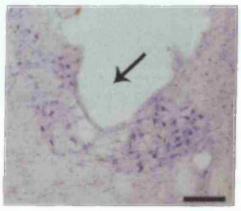
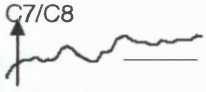
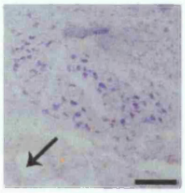
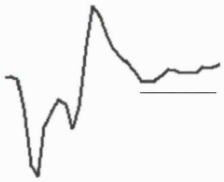
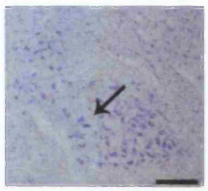
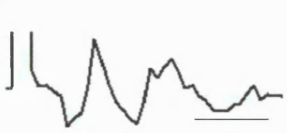
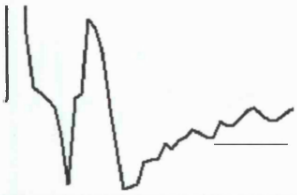
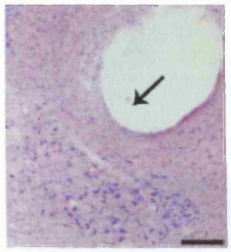
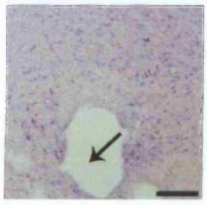
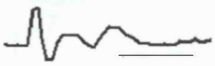
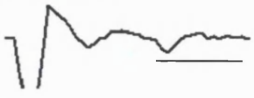
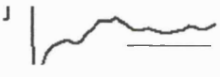
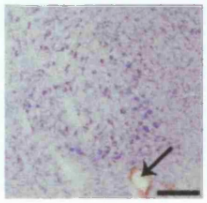
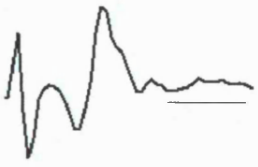
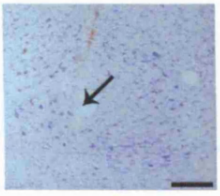
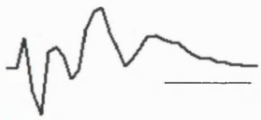
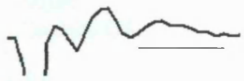
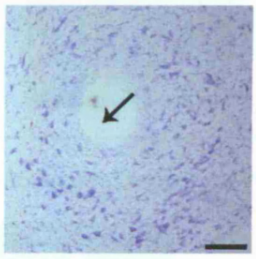
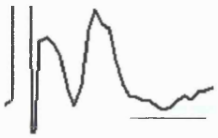
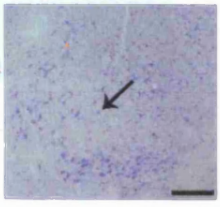
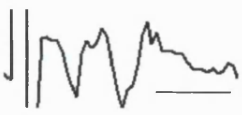
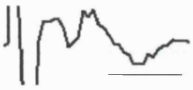
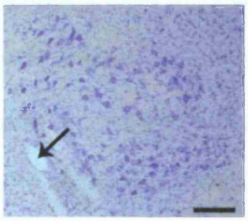
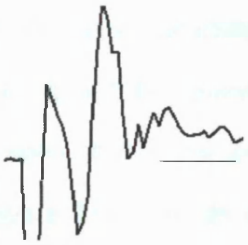
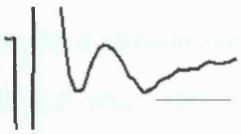
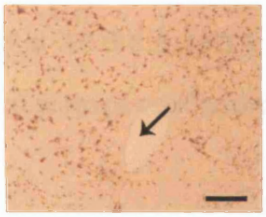
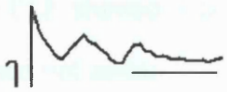
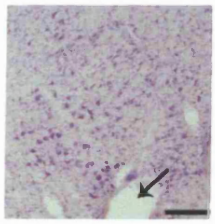
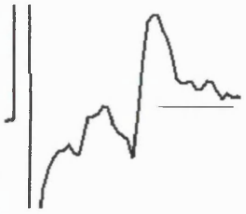
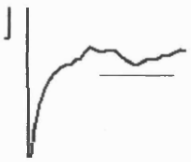
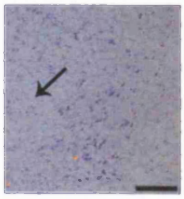
Exp. No.	Stimulation site- Distance from caudal edge of RN of (μm)	Contra CDP	Ipsi CDP
R524	-100 / mid RNm 	60μA Caudal C7 	60μA Caudal C7 
R533	-100 / Caudal pole of RNm 	30μA Rostral C6 	30μA Rostral C6 
R474	-100 / mid RNm 	30 μA Rostral C3  C5/C6  C7/C8 	Not sampled
R550	-200 / ventral to RNm 	50μA Caudal C7 	50μA Caudal C7 

Table 2.1 Summary of stimulation sites within the midbrain and their corresponding cord dorsum volleys. At the end of all experiments, a micro-lesion was produced in the red nucleus (RN) to allow histological confirmation of the stimulation site. Photomicrographs show the lesion indicating the stimulation site in the RN and the distance from caudal edge of the RN is stated. - indicates rostral + indicates caudal. Results appear in a caudal to rostral order. Scale bar in traces is 1ms, and in micrographs 200μm. Arrows in R474 indicate onset time of stimulus artefact.

Exp. No.	Stimulation site- Distance from caudal edge of RN of (μm)	Contra CDP	Ipsi CDP
R619	-300/mid RNm 	40μA C6/C7 	40μA C6/C7 
R478	-300 / dorsal to RN 	100μA C6 	100μA C6 
R511	-400 / ventral aspect of RNm 	70μA Rostral C3  Caudal C6 	70μA Caudal C6 
R432	-400 / Ventral to RNm 	50μA Rostral C3  C5/C6  Caudal C7 	50μA Rostral C3  C5/C6  Caudal C7 

Exp. No.	Stimulation site- Distance from caudal edge of RN of (μm)	Contra CDP	Ipsi CDP
R506	-400 / ventral to RNm 	50μA Rostral C6 	50μA Rostral C6 
R627	-450 / Mid RNm 	50μA Caudal C3  Rostral C6  C7/C8 	50μA Caudal C3  Rostral C6  C7/C8 
R536	-600 / mid RN 	50μA C7 	50μA C7 
R475	-600 / mid RN 	65μA C5/C6 	65μA C5/C6 

Exp. No.	Stimulation site- Distance from caudal edge of RN of (μm)	Contra CDP	Ipsi CDP
R438	-600 / medial & ventral to RNm 	40μA C7/C8 	40μA C7/C8 
R410	-600 / lateral and ventral aspect of RNm/RNp 	200μA C6 	200μA C6 
R445	-600 / lateral & ventral to RNm 	50μA Caudal C7 	50μA Caudal C7 
R521	-950/rostral aspect of RN 	75μA C7 	75A C7 

The cumulative data illustrated in table 1 can be confirmed from data in a single animal. Figure 2.4 shows recordings obtained in one experiment where the rostro-caudal location of the stimulation electrode in the midbrain was varied. From this and from the data shown in table 1, a common pattern can be discerned from which an early volley is seen both in the contralateral and ipsilateral DLF when stimulating the caudal part of the RNm (AP2.96 in figure 2.4). However, even with a stimulus current of 200μA, an early volley was seen only in the ipsilateral DLF when stimulating caudal to the RNm (AP1.96 in figure 2.4). The late volley was seen only within the contralateral DLF and was larger in amplitude when stimulating the more rostral part of the RNm (AP2.96-3.4 in figure 2.4). In this particular experiment illustrated in figure 2.4, and at AP 3.8 & AP3.4, recording from the ipsilateral DLF showed a positive deflection perhaps corresponding to an early volley but this was not usual.

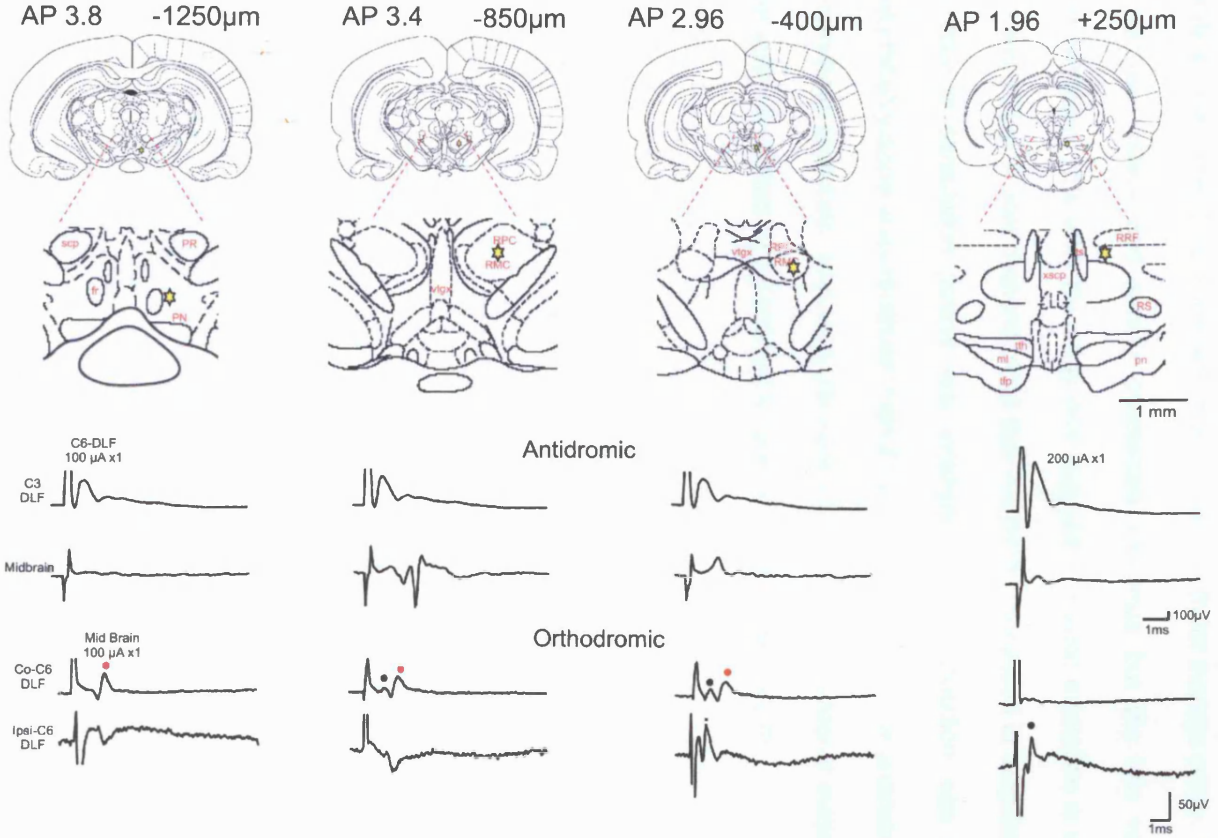


Figure 2.4 Location of stimulation sites giving maximal amplitude for early and late volley. The top pair of rows are illustrations taken from Paxinos' atlas indicating the stimulation site within the midbrain (☆). The anterior-posterior coordinates are indicated and the distance from the caudal edge of the RN is stated. + is caudal to the caudal edge and – is rostral to caudal edge of RN. The 2nd pair of rows shows antidromic action potentials recorded at the marked sites together with the corresponding cord dorsum potentials. The 3rd pair of rows shows the orthodromic cord dorsum potentials recorded at C6 for the contralateral (co-C6 DLF) and ipsilateral (Ipsi-C6 DLF) spinal cord. In this experiment, the largest antidromic potentials were seen at AP 3.4, which corresponds to the rostral part of the magnocellular red nucleus (RN). Stimulation rostral in the RN (AP 3.8 and AP 3.4) produced a late descending volley in the contralateral cervical spinal cord (●). Stimulation more caudal (AP 2.96) produced, in addition to the late volley, an early descending volley, seen in both sides of the cord (●). Stimulation further caudal (AP 1.96) disclosed only the ipsilateral volley. The electrical threshold and conduction velocities of these volleys were similar. *Fr fasciculus retroflexus ml medial lemniscus PN paranigral nucleus pn pontine nuclei PR prerubral field RMC magnocellular red nucleus RPC parvicellular red nucleus RRF retrorubral field RS rubrospinal tract scp superior cerebellar peduncle tfp transverse fibres of pons ts tectospinal tract tth trigeminothalamic tract vtgx ventral tegmental decussation xscp decussation superior cerebellar peduncle*

The peak negativity of the early volley usually occurred at about 1ms from the onset of the stimulation artefact and the peak negativity for the late one usually occurred approximately 1ms after the first one. In order to calculate the conduction velocities, the latencies for both the early and the late volleys were plotted against the distance along the cervical cord, as demonstrated in Figure 2.5. A line of best fit for each plot was taken and the conduction velocity was calculated as the inverse slope of the linear regression. The two descending volleys generally had similar conduction velocities and thresholds. The average conduction velocity for the early volley was $44.6ms^{-1} \pm 2.2$ (n=11) and was $49.7ms^{-1} \pm 1.9$ (n=10) for the late volley. The fact that these two volleys had similar conduction velocities but the late volley arrived approximately 1ms after the early one suggests synaptic excitation is involved with the late volley. It was demonstrated that this volley increased in amplitude when the number of stimulation pulses was increased. This facilitation was observed in Ketamine/xylazine anaesthetised animals and was not evident in animals under other anaesthetic protocols. This temporal facilitation provides additional evidence that the late volley is elicited by synaptic activation of neurons (figure 2.6).

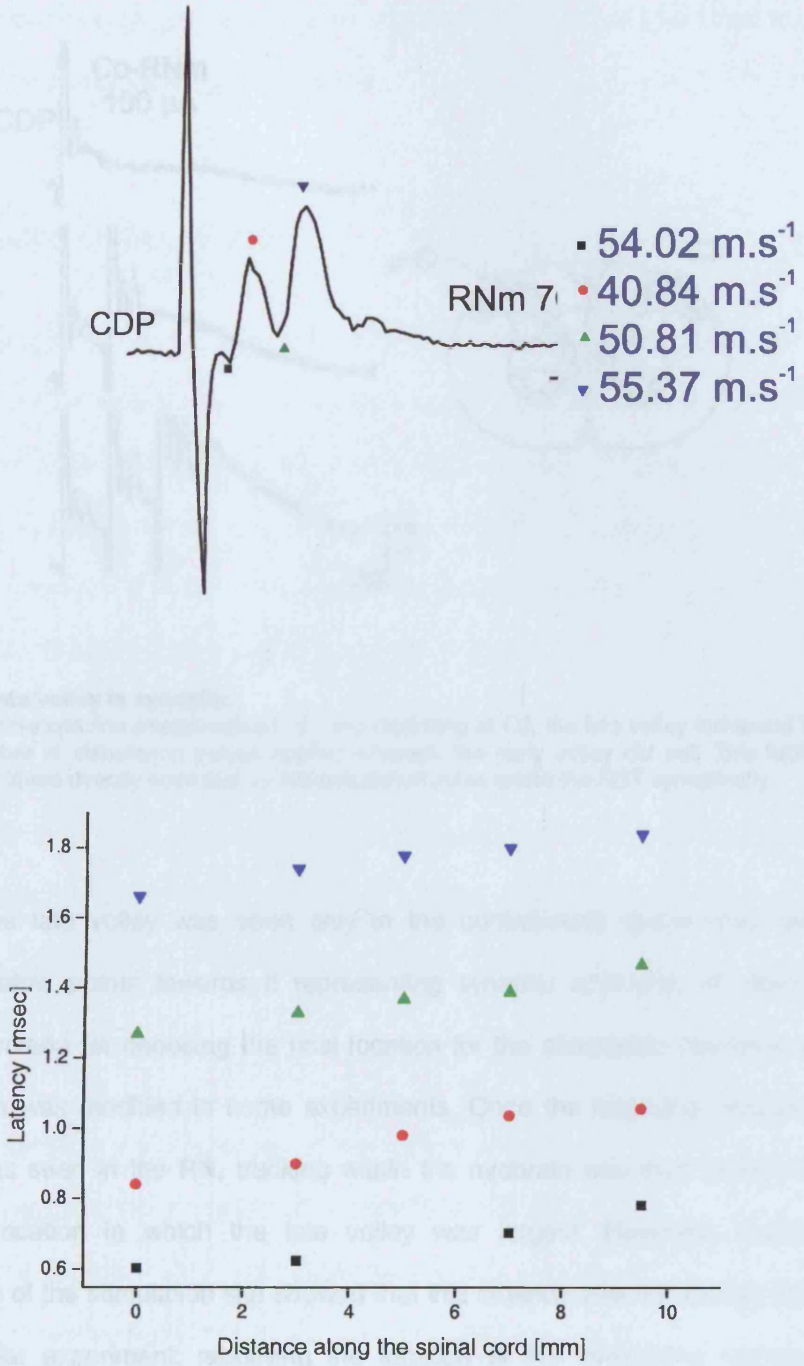


Figure 2.5 Conduction velocities of R511.
Along the spinal cord, latencies were measured from the onset of the stimulation artefact to the indicated deflections in the CDP recording. The conduction velocities were then calculated as the inverse of the linear regression.

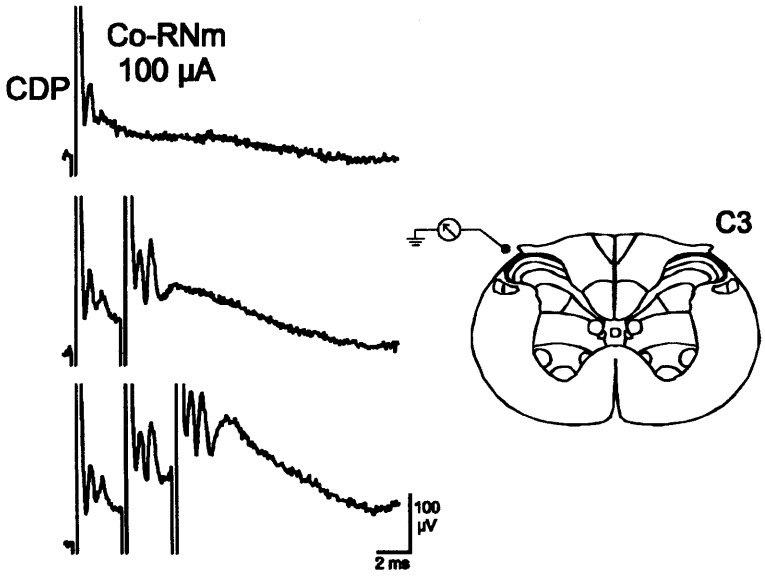


Figure 2.6 Late volley is synaptic.
From a ketamine/xylazine anaesthetised rat, and recording at C3, the late volley increased in size with the number of stimulation pulses applied whereas the early volley did not. This facilitation suggests that fibres directly activated by the stimulation pulse excite the RST synaptically.

Because the late volley was seen only in the contralateral spinal cord and the evidence below points towards it representing synaptic activation of rubrospinal fibres, the criteria for choosing the final location for the stimulation electrode within the midbrain was modified in some experiments. Once the largest antidromic field potential was seen in the RN, tracking within the midbrain was then carried out to verify the location in which the late volley was largest. However, histological confirmation of the stimulation site showed that this criterion was not always ideal. In one particular experiment, modifying the location of the stimulating electrode in accordance to the observance of a large late volley resulted in the final electrode location being dorsal to the RN (see R478 in table 1). As a result, other fibres other than of the RST may have been activated (see intraspinal maps). Therefore, although a late volley may indicate synaptic activation of the RST, if selective activation of the RST is derived, a large antidromic response should also be

observed at the chosen site and a small stimulus current should be used to avoid activation of other pathways (see discussion).

2.3.2 Intraspinal recordings

Intraspinal recordings were carried out to investigate the distribution of the synaptic actions of the RST within the caudal cervical spinal cord. Isopotential maps of field potential recordings were produced in seven control animals. A volley corresponding to the late volley in the CDP was seen in the contralateral spinal cord within the dorsolateral funiculus in 5 out of 7 animals and the average amplitude for this volley was 119.7μV. A volley corresponding to the early volley in the CDP was seen in the contralateral DLF in 5 out of 7 animals (average amplitude 121.5 μV) and was seen in the ipsilateral DLF in 2 out of 7 animals (average amplitude 149.7μV). Usually, the synaptic potentials were seen within the intermediate grey of the contralateral spinal cord. However, 3 out of 7 animals showed synaptic potentials within the ipsilateral spinal cord. The maximum amplitude of these potentials ranged from 50-212μV (average 128.9μV).

In order to calculate the amplitudes of the volleys and synaptic potentials, a cursor was located at the time of maximum amplitude and another one was located at a reference time (Figure 2.7). A late and/or early volley was seen in the DLF (Figure 2.7, A), but in some experiments, as the tracks approached the grey matter the shape of the volley resembled a terminal potential (Figure 2.7, B). This would explain why in some experiments, the focus for the volley extended into the grey matter as the cursor locations would measure the amplitude for the volley but would then measure the amplitude for the terminal potentials. If two volleys were seen in the field potential recordings (FP), then both volleys may contribute to the synaptic potential.

Figure 2.7, C shows a synaptic wave in which the amplitude was measured at the marked cursors (dotted lines). However, responses which occurred at an earlier latency were also seen (arrow). It is often difficult to determine whether these earlier responses represent a volley corresponding to the early volley in the CDP or presynaptic responses. Figure 2.7, D shows an example taken from an experiment in which only a volley corresponding to the early volley in the CDP was seen. Here, a synaptic potential is seen, and preceding it is a terminal potential (arrow). As can be seen, stimulation of the RN often produces two volleys and synaptic potentials often consisting of terminal potentials.

Table 2 shows a summary of the presence of the volleys as recorded from the cord dorsum and from the intraspinal recordings. The stimulus current used in the intraspinal experiments ranged from 35μA to 100 μA in (see maps). A small stimulus intensity was used when possible which generally depended on the location of the electrode within the RN and the observance of volleys of a reasonable amplitude. The ticks represent the presence of a late and/or early volley in the cord dorsum (✓) and the presence of a late and/or early volley and synaptic potentials in the intraspinal records (✓).

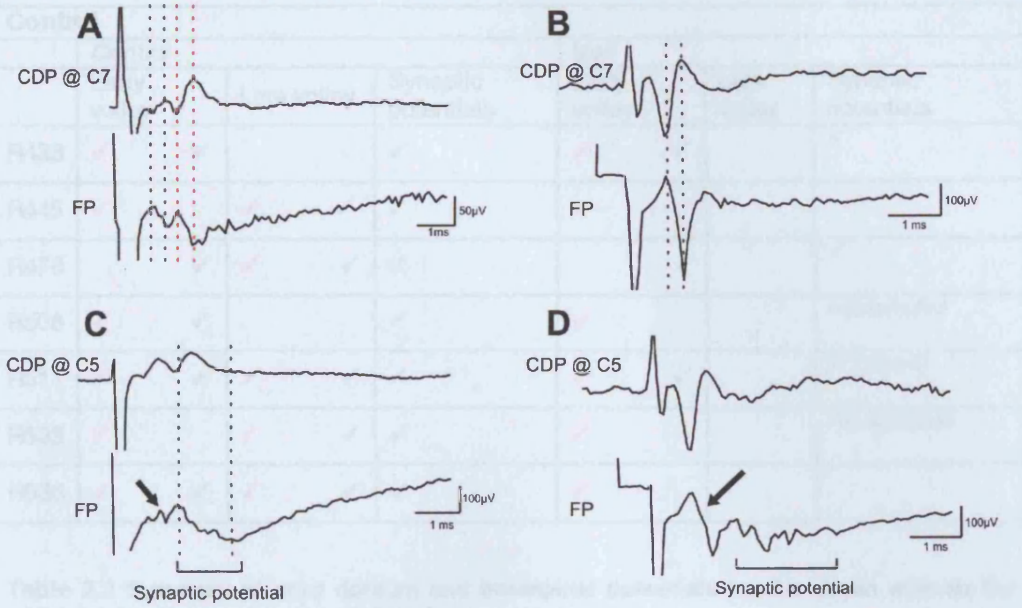


Figure 2.7 Intraspinal records.
Intraspinal recordings (FP) where made in the caudal cervical spinal cord while stimulating the contralateral red nucleus (A-D from different animals). **A** Recordings made within the contralateral DLF shows two volleys corresponding to the early and late volley seen in the CDP. Dotted lines represent the latencies at which the amplitude was measured for the early volley (black) and the late volley (red), **B** Recordings made in the white/grey matter border showing a terminal potential, **C** Synaptic potential recorded from the intermediate grey of the contralateral spinal cord. Responses also occurred at an earlier latency (arrow) probably mediated by the early volley. **D** Synaptic potential recorded from the intermediate grey of the contralateral spinal cord. The synaptic potential here is preceded by what looks like a terminal potential (arrow).

Control						
	Contra			*Ipsi*		
	Early volley	Late volley	Synaptic potentials	Early volley	Late volley	Synaptic potentials
R438	✓ ✓		✓	✓ ✓		✓
R445	✓	✓ ✓	✓	✓		
R478	✓	✓ ✓	✓	✓		✓
R506	✓ ✓		✓	✓		not sampled
R511	✓ ✓	✓ ✓	✓	✓ ✓		✓ (small)
R533	✓	✓ ✓	✓	✓		not sampled
R536	✓ ✓	✓ ✓	✓	✓		

Table 2.2 Summary of cord dorsum and intraspinal potentials for the seven animals for which intraspinal maps were produced.
Stimulation of the red nucleus often produced two descending volleys (early and late) in the contralateral spinal cord and one volley in the ipsilateral spinal cord (early). ✓ Refers to cord dorsum potentials ✓ Refers to intraspinal potentials

Correlation of the contralateral synaptic potentials to the late volley

Grading of the stimulus intensity in a number of experiments demonstrated that the contralateral synaptic potentials could be mediated by the late volley alone, which itself is thought to be mediated by synaptic activation of rubrospinal fibres (see discussion). Figure 2.8 demonstrates how increasing the stimulus intensity in the red nucleus produced a similar increase in amplitude in both the late volley and the recorded synaptic potentials. In this particular example, taken from R445, there is a clear difference in the threshold between the two volleys, allowing a clear separation of their effects. The early volley had a relatively high threshold, and was small in amplitude even at an intensity of 100μA. On the other hand, the second volley had a lower threshold, and the correlation between this late volley and the synaptic potentials is clearly demonstrated in this figure.

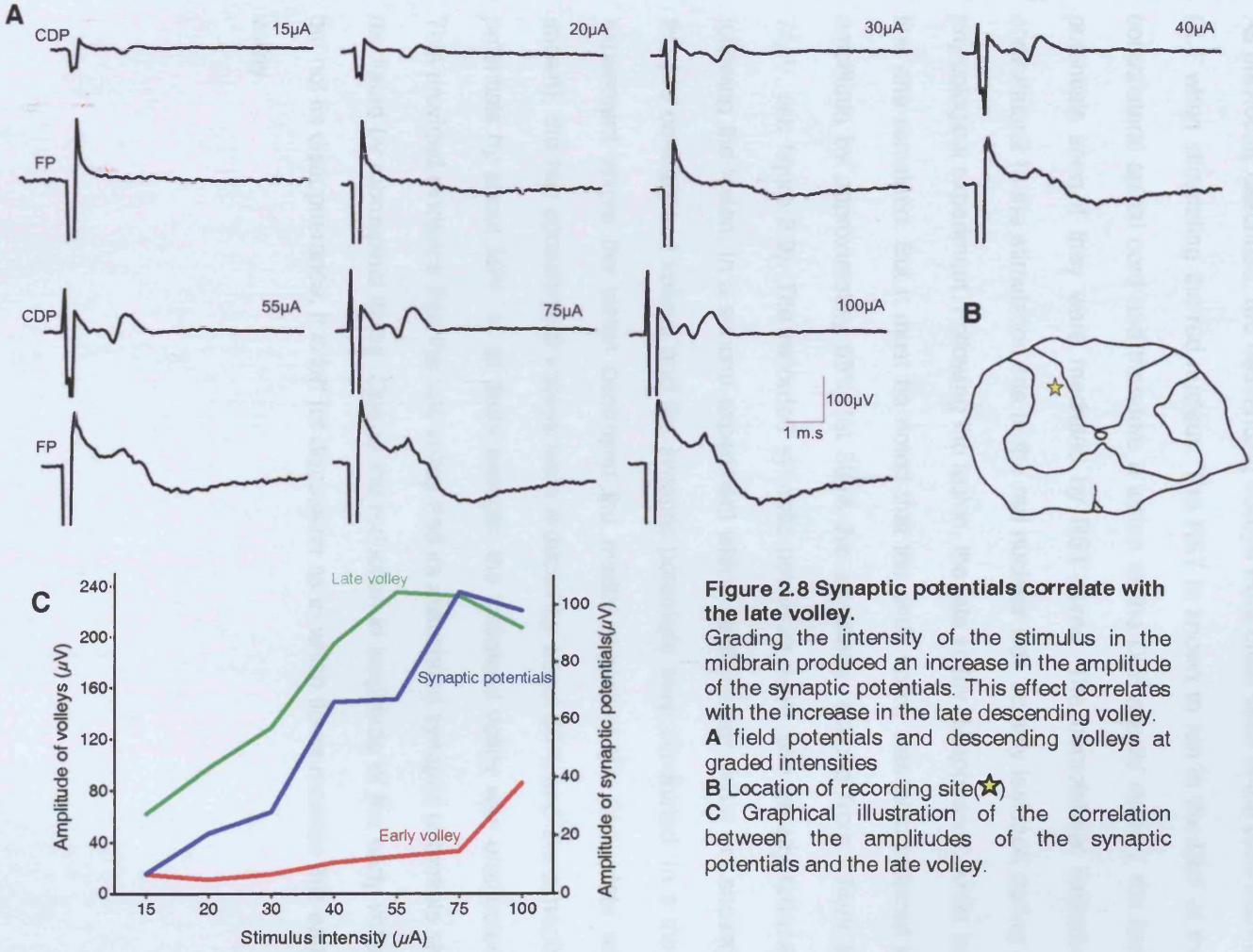
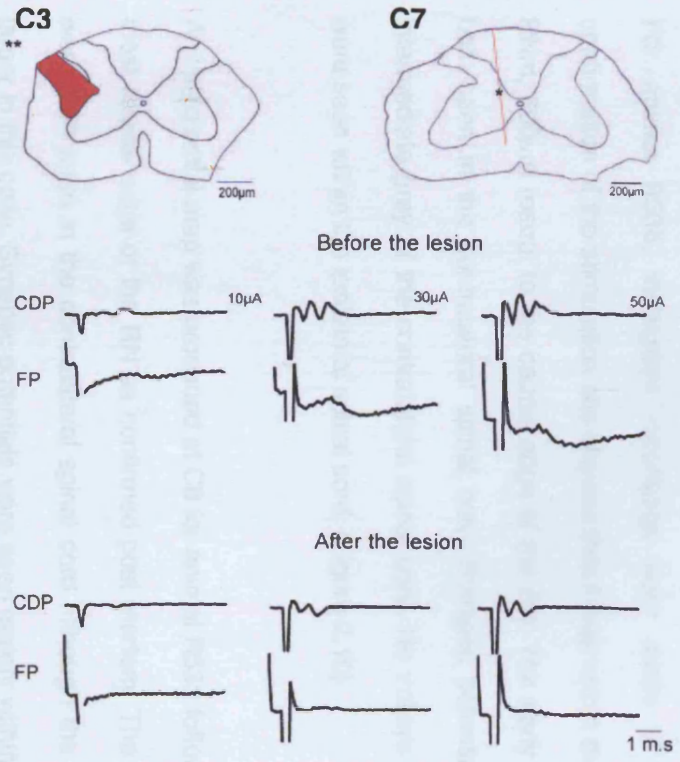
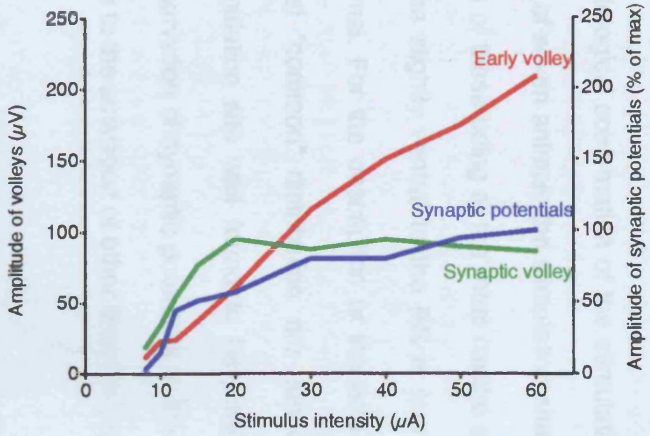


Figure 2.8 Synaptic potentials correlate with the late volley.
Grading the intensity of the stimulus in the midbrain produced an increase in the amplitude of the synaptic potentials. This effect correlates with the increase in the late descending volley.
A field potentials and descending volleys at graded intensities
B Location of recording site(☆)
C Graphical illustration of the correlation between the amplitudes of the synaptic potentials and the late volley.

The late volley and synaptic potentials disappear following an acute lesion to the DLF

As previously described, two descending volleys were often seen in the contralateral DLF when stimulating the red nucleus. The RST is known to run in the DLF of the contralateral spinal cord and therefore a lesion to the DLF should destroy the field potentials seen if they were mediated by RST fibres. The dorsolateral funiculus contralateral to the stimulation site in the red nucleus was acutely lesioned during a physiological experiment. Following the lesion, the late volley disappeared, while the first one remained. But it must be noted that the early volley was also reduced in amplitude by approximately 55% (at 50μA the amplitude reduced from 174μV to 74μV, see figure 2.9). The recorded synaptic potentials were also greatly reduced following the lesion. In a second experiment with a similar lesion (data not shown), the two contralateral volleys and the synaptic potentials were eliminated. In a third experiment where the lesion destroyed the medial aspect of the DLF (data not shown), the two contralateral volleys were reduced by about 50% and the synaptic potentials by about 80%. In all three animals, the ipsilateral volley was unaffected. This provided evidence that the late volley and its associated synaptic potentials are mediated by rubrospinal fibres. Due to the reduction in amplitude of the early volley but not its disappearance, it is left for discussion as to which fibres mediate this early volley.





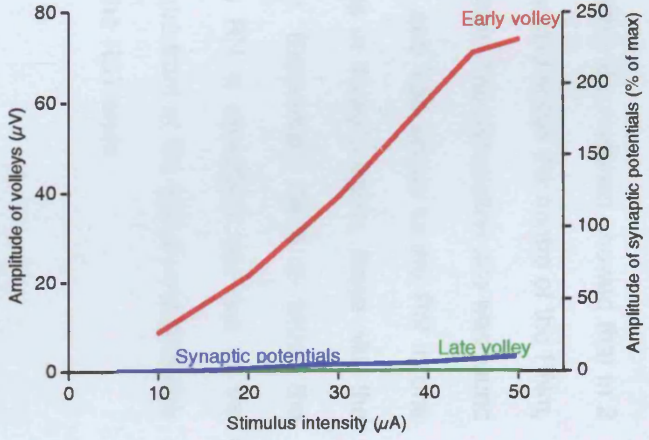


Figure 2.9 Synaptic potentials are greatly reduced following an acute lesion to the DLF.
Field potentials (FPs) representing synaptic excitation were recorded in the intermediate zone of the contralateral cervical spinal cord caudal to the lesion (**) and were of a latency appropriate to a direct effect of the late volley. Recording site for FPs is indicated (*). Following an acute lesion to the DLF, the second volley disappears and the synaptic potentials were also reduced confirming that the late volley and the synaptic potentials associated with it represent rubrospinal actions. Red line represents the electrode track

Description of synaptic potentials

Histological confirmation of the stimulation site within the midbrain showed that in 2 out of seven animals the stimulation sites were located within the centre of the RNm, one of these being at the most caudal edge of RNm. The stimulation site was found to be slightly ventral to the RN in four animals and was dorsal to the RN in one animal. For the description of the field potentials in these animals, those with the most "common" distribution are described first. Experiment R478 in which the stimulation site was found to be dorsal to the RN is described last due to the observation of synaptic potentials in the ventral quadrant of the spinal cord probably due to the activation of other fibres in addition to the RST ones.

In the following descriptions, volleys described are those seen in the intraspinal records that correspond to the early and/or late volley seen in the cord dorsum recording.

For animal R536, intraspinal recordings were made at C7 and histological confirmation of the stimulation site showed that it was within the medial aspect of the RNm, 600μm rostral to the caudal edge of the RN. The early and late volleys were both seen in the contralateral spinal cord. Synaptic potentials were seen in the intermediate grey of the contralateral spinal cord. No volleys or synaptic potentials were seen within the ipsilateral spinal cord. (Figure 2.10)

An isopotential map was produced at C8 for animal R533 following stimulation of the most caudal edge of the RN as confirmed post mortem. The early and late volleys were both seen in the contralateral spinal cord although the late volley was much larger in this case. Synaptic potentials were seen mainly within the intermediate grey of the contralateral spinal cord. However, the isopotentials map also shows spread of

negativity towards the most dorsolateral motor column (lamina IX). In this particular experiment, potentials in the ipsilateral spinal cord were not sampled so it cannot be confirmed whether ipsilateral synaptic potentials were present (Figure 2.11).

The third animal showing the most common distribution of synaptic potentials is R445 with a CDP also dominated by the late volley. The isopotential map for this animal was produced with a double stimulus to the RN at C6. Histological confirmation of the stimulation site demonstrated that it was within the lateral and ventral aspect of the RNm, 600μm rostral to the caudal edge of the RN. A late volley was seen in the contralateral spinal cord within the lateral border of the DLF and dorsal horn. No early volley was seen in the contra- or ipsilateral spinal cord. Synaptic potentials were seen within the intermediate grey of the contralateral spinal cord (Figure 2.12). Note, the late latency of the synaptic potentials is clear here, appropriate to an origin in the late volley (cf. figure 2.8 taken from the same animal showing a correlation of the synaptic potentials to the late volley).

Figures 2.10-2.15 & 2.17 **Field potential maps for control animals.** The electrophysiological actions of the RST in the caudal cervical spinal cord of control animal are illustrated in isopotential maps. Photomicrographs of the final stimulation sites in the midbrain (MB) are shown. If antidromic field potentials were recorded at the final stimulation site, the potentials are illustrated. The descending volleys and synaptic potentials are illustrated. The latencies at which the amplitudes for these potentials were measured are indicated by the dotted lines. The same calibration bar (-400 to +400μV) is used for all figures except for R533, R536, and R601.

Figure 2.10 R536

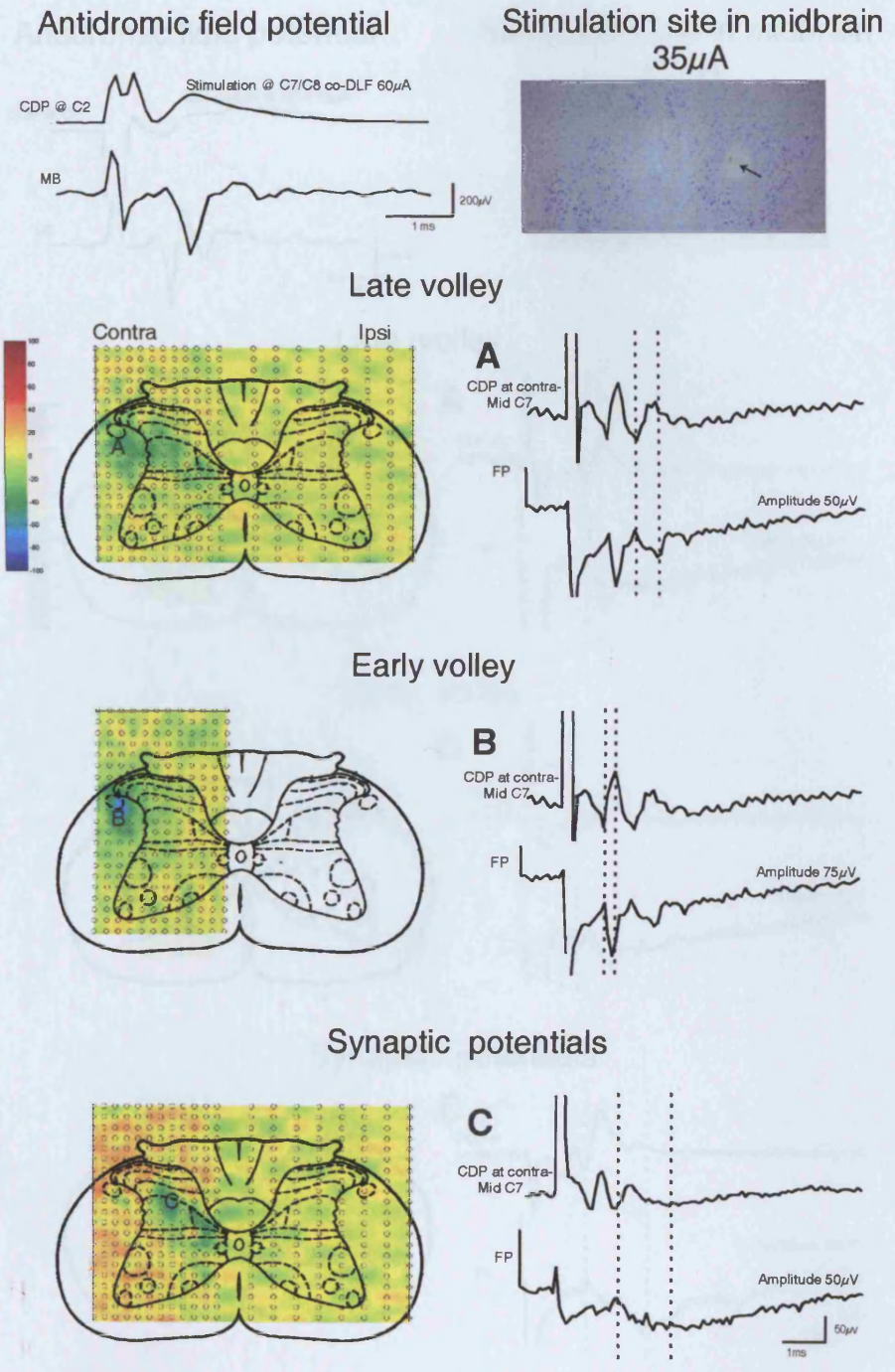


Figure 2.11 R533

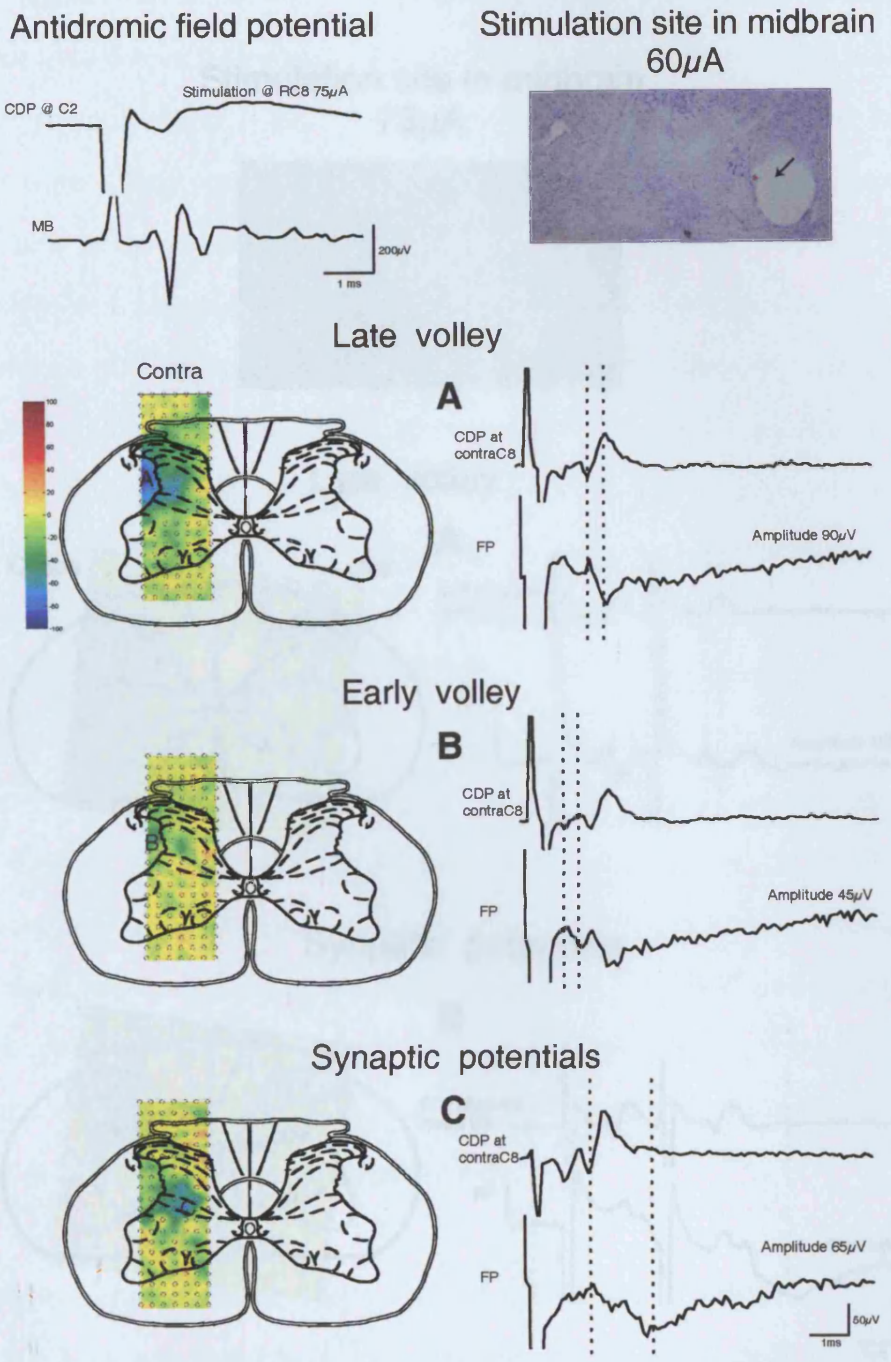
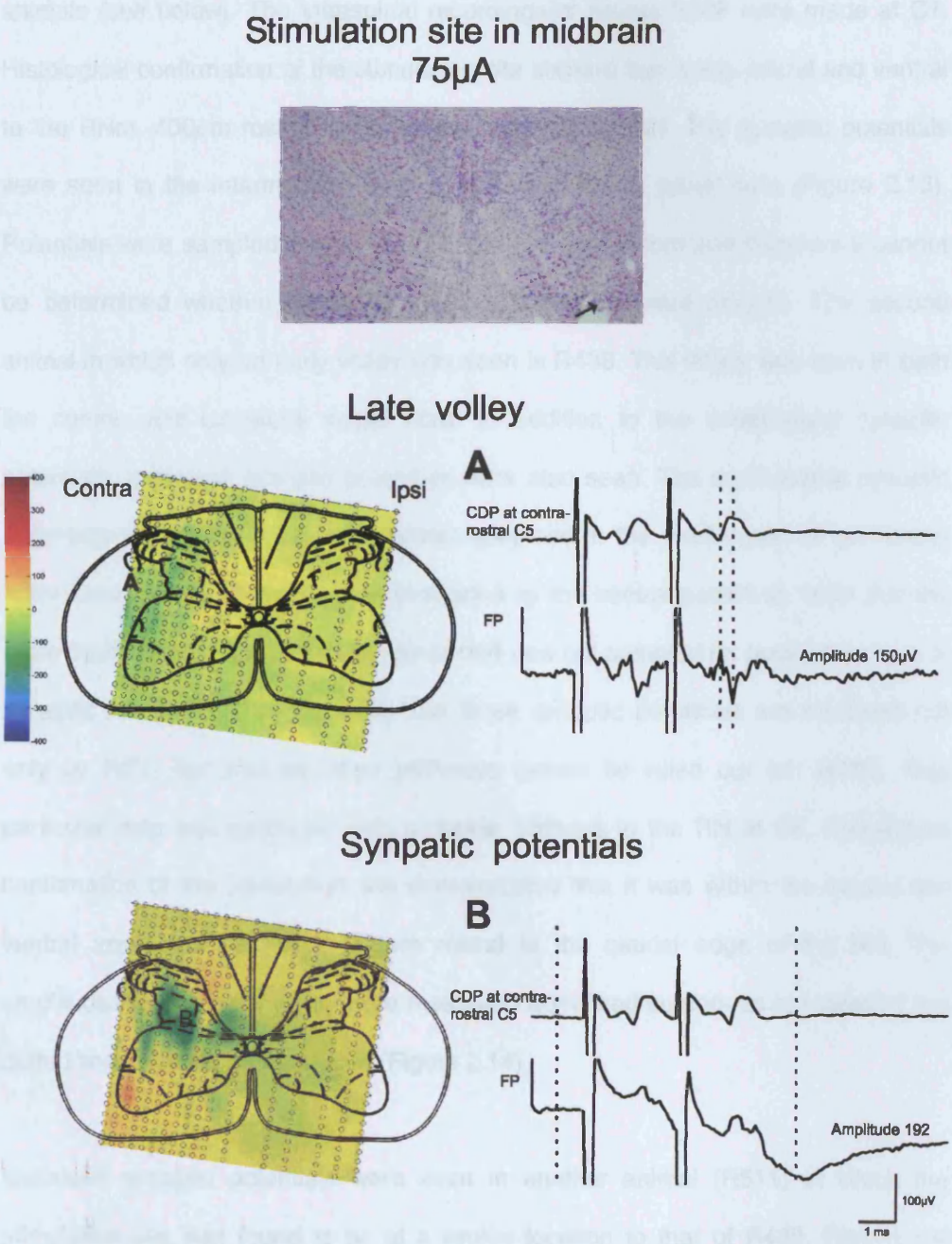


Figure 2.12 R445



Although a late volley was usually seen in the contralateral spinal cord, this was not the case in two animals. Only an early volley was seen in both R506 and R438 in which the stimulation site was found to be at a similar location in the RN for both animals (see below). The intraspinal recordings of animal R506 were made at C7. Histological confirmation of the stimulation site showed that it was lateral and ventral to the RNm, 400μm rostral to the caudal edge of the RN. The synaptic potentials were seen in the intermediate grey of the contralateral spinal cord (Figure 2.13). Potentials were sampled only in the contralateral spinal cord and therefore it cannot be determined whether ipsilateral synaptic potentials were present. The second animal in which only an early volley was seen is R438. This volley was seen in both the contra- and ipsilateral spinal cord. In addition to the contralateral synaptic potentials, ipsilateral synaptic potentials were also seen. The contralateral synaptic potentials were seen in the intermediate grey and in the medial part of the ventral horn (lamina VII & VIII), but were dominated by the ventral potentials. Note that the more medial part of the ipsilateral spinal cord was not sampled for possible volleys or synaptic potentials. The possibility that these synaptic potentials are mediated not only by RST, but also by other pathways cannot be ruled out (cf. R478). This particular map was produced with a double stimulus to the RN at C6. Histological confirmation of the stimulation site demonstrated that it was within the medial and ventral aspect of the RNm, 600μm rostral to the caudal edge of the RN. The amplitudes for the early volley were measured from fixed cursors as indicated by the dotted lines marked on the traces (Figure 2.14).

Ipsilateral synaptic potentials were seen in another animal (R511) in which the stimulation site was found to be at a similar location to that of R438. Histological confirmation of the stimulation site showed that it was within the medial aspect of the RNm and slightly ventral and was 400μm rostral to the caudal edge of the RN. Intraspinal recordings were made at C5. A late volley was seen in the contralateral

DLF and an early volley was seen in both the contra- and ipsilateral DLF, but was much larger in amplitude in the ipsilateral DLF (Figure 2.15, illustrated). Note that the early volley in R511 in the ipsilateral CDP was not particularly large (table 3). The synaptic field potentials were seen mainly within the intermediate grey of the contralateral spinal cord. However, small synaptic potentials were seen in the ipsilateral spinal cord within the intermediate grey (Figure 2.16)

Figure 2.13 R506

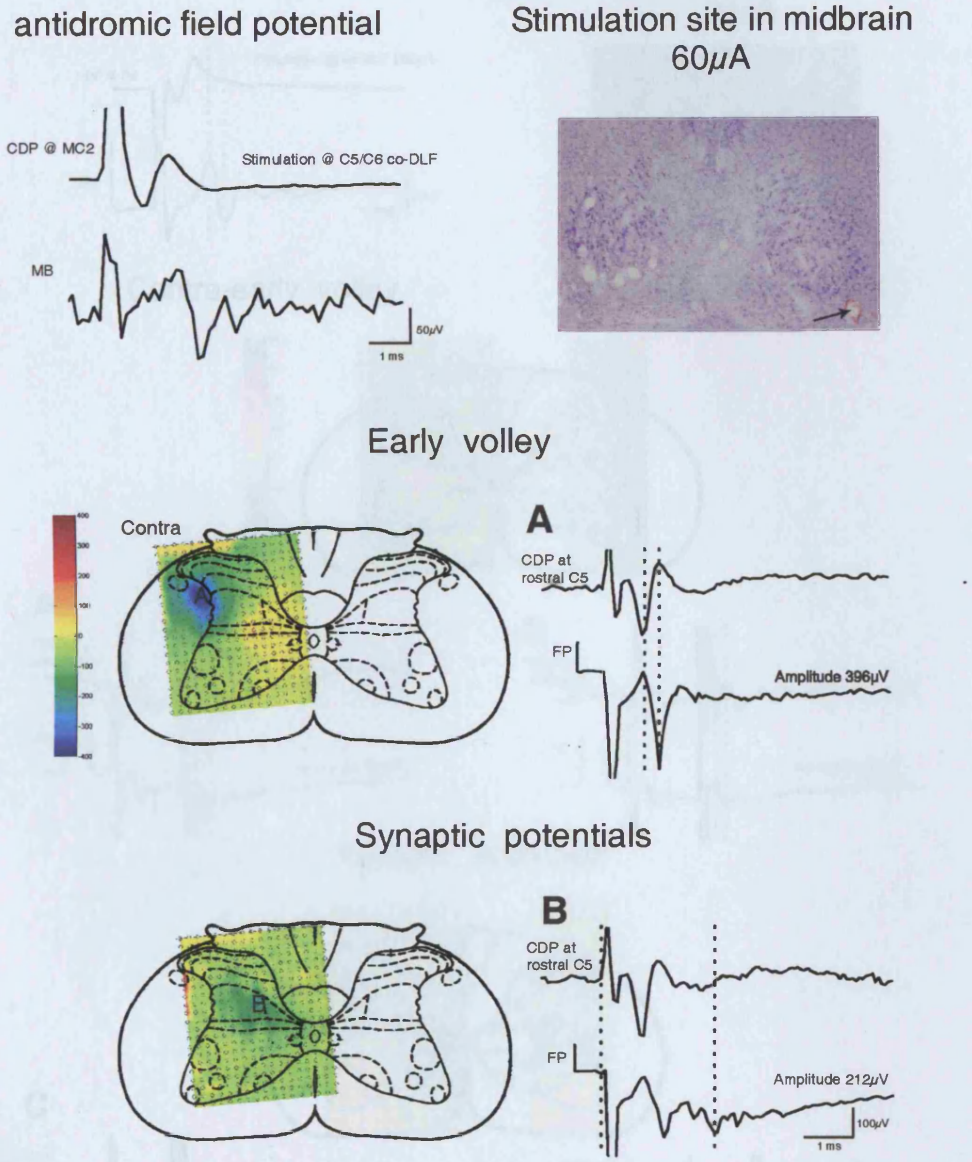


Figure 2.14 R438

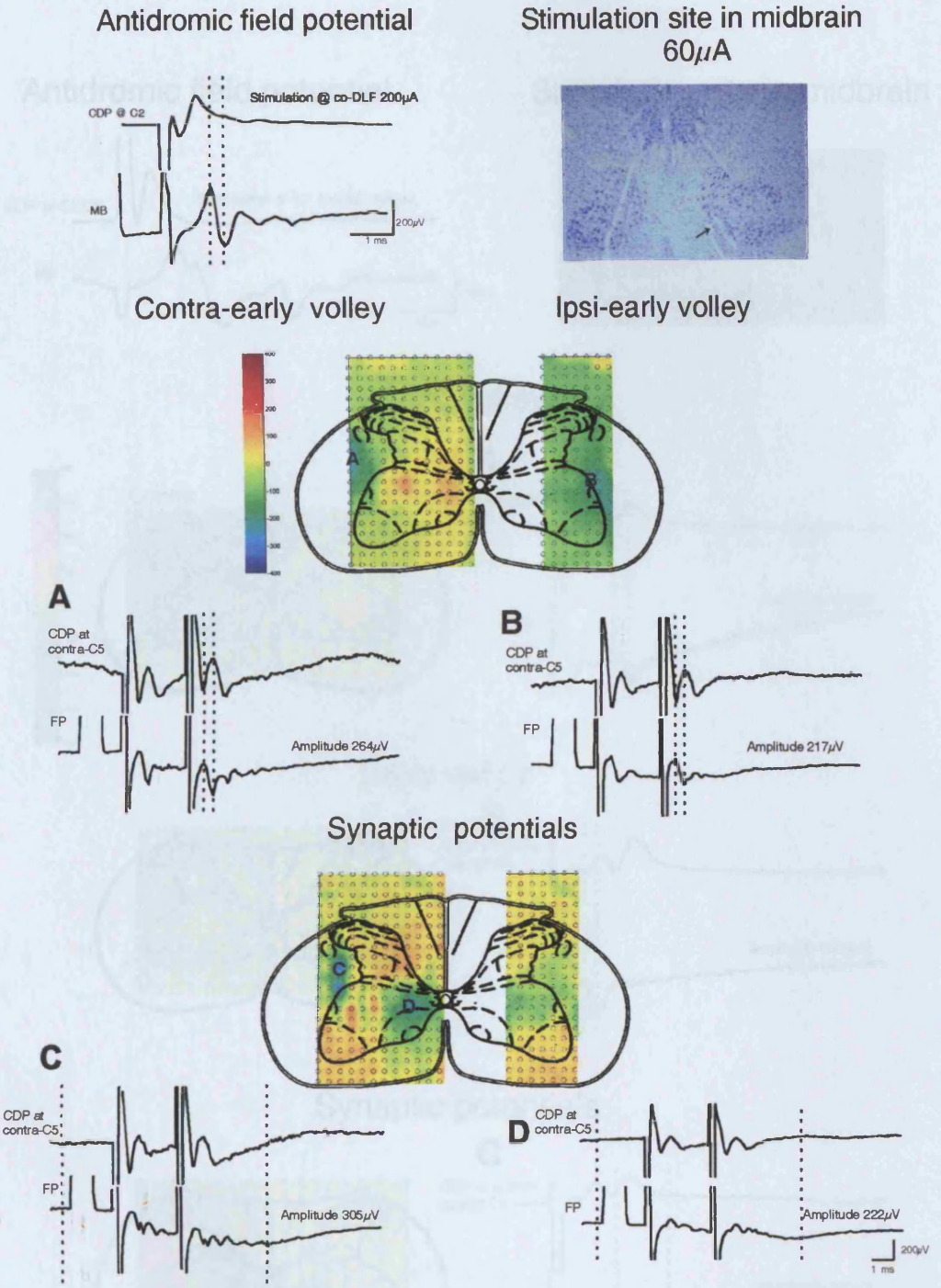
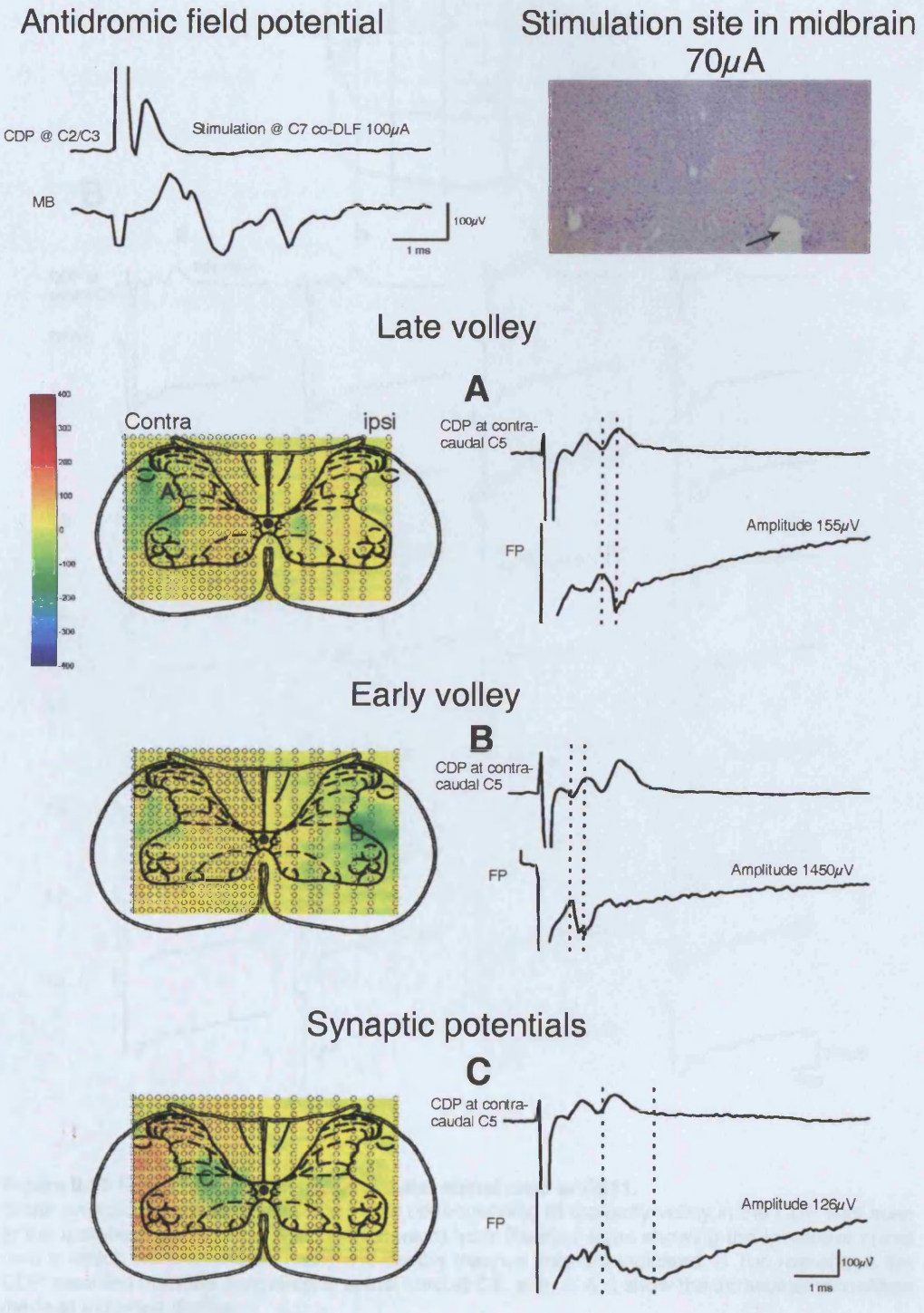


Figure 2.15 R511



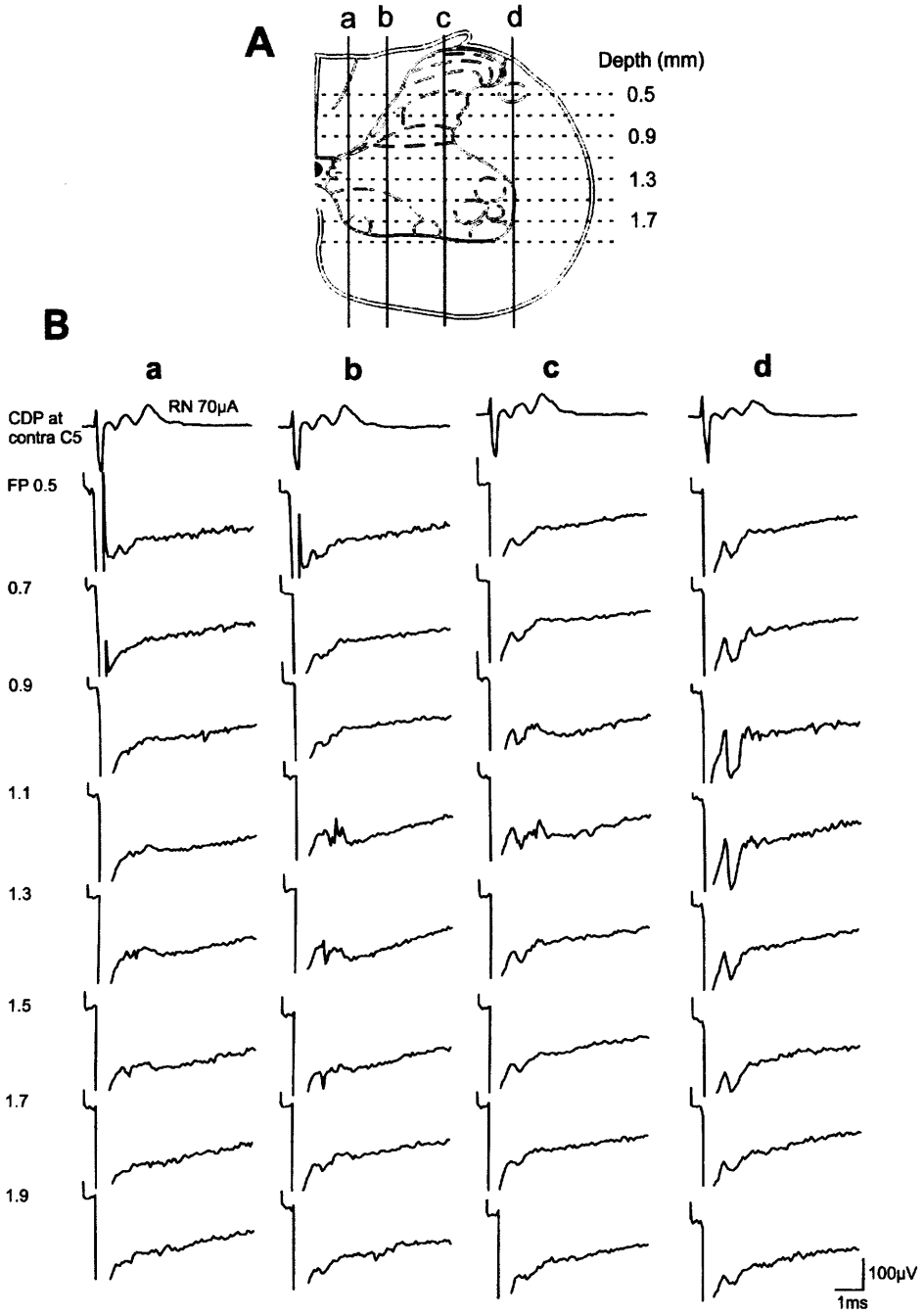
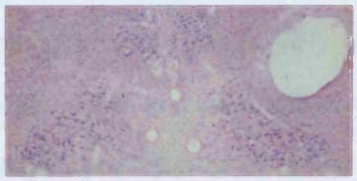


Figure 2.16 Field potentials in the ipsilateral spinal cord of R511.
Small synaptic potentials and a large volley corresponding to the early volley in the CDP was seen in the ipsilateral spinal cord. A Illustration taken from Paxinos' atlas showing the ipsilateral spinal cord in which the tracks (solid lines) and depths (dashed line) are indicated. B Top row shows the CDP recorded from the contralateral spinal cord at C5. a, b, c, & d show the intraspinal recordings made at indicated depths.

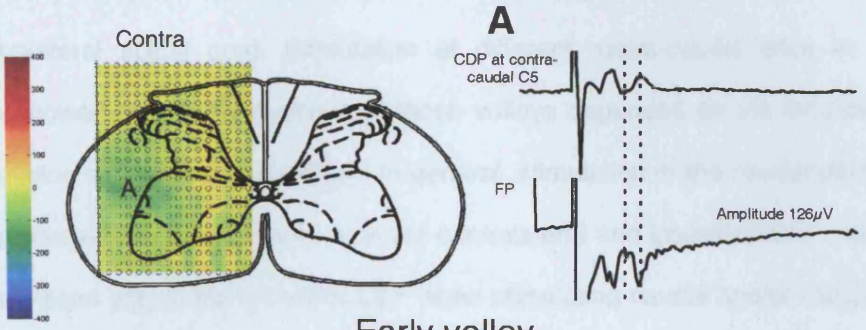
Finally, animal R478 is an example for which the final location of the stimulation site in the midbrain was adjusted to produce a large late volley. This resulted in the final location being dorsal to the red nucleus. Presumably, as a result, small synaptic potentials were seen in the intermediate grey and much larger ones in the ventral quadrant of the spinal cord, both ipsilaterally and contralaterally. Histological confirmation of the stimulation site showed that it indeed was dorsal to the RNm, 300μm rostral to the caudal edge of the RN. The map for this animal was produced at C6. In the contralateral spinal cord, both a late and an early volley were seen within the DLF (Figure 2.17). A small early volley was seen in the ipsilateral spinal cord but was unitary and dispersed and the amplitudes therefore could not be measured at a fixed latency. Because the final location of the electrode was not where a maximum antidromic potential was seen, an antidromic action potential is not illustrated in Figure 2.17.

Figure 2.17 R478

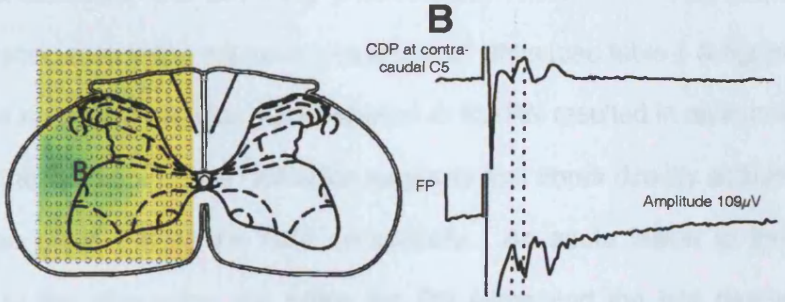


Stimulation site in midbrain
100μA

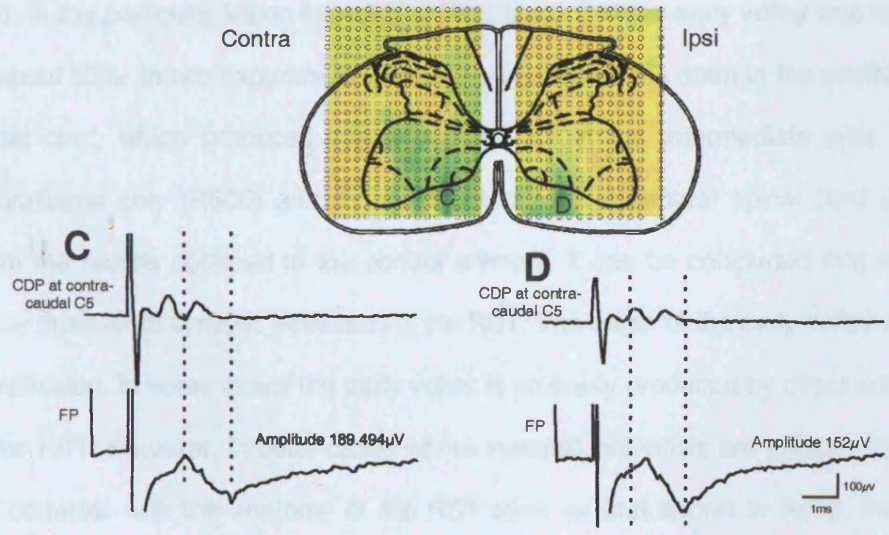
Late volley



Early volley



Synaptic potentials



2.3.3 Summary

Stimulation of the caudal cervical spinal cord produced antidromic field potentials in the contralateral red nucleus (RN). Simulation of the RN produced distinct potentials in both the contra- and ipsilateral cervical spinal cord. In the contralateral spinal cord, two volleys were often seen, a late one and an early one. In the ipsilateral spinal cord, an early volley was often seen with a similar latency to the early volley seen in the contralateral spinal cord. Stimulation at different rostro-caudal sites in the midbrain showed that the occurrence of these volleys depended on the location of the stimulation site within the midbrain. In general, stimulation in the caudal parts of the RN produced an early volley in both the contralateral and ipsilateral DLF, and is sometimes seen only in the ipsilateral DLF when stimulating caudal and/or ventral to the RNm. The late volley was seen only within the contralateral DLF and was larger in amplitude when stimulating the more rostral part of RNm (see table 1 & figure 2.4). Increasing the number of stimulus pulses applied to the RN resulted in an increase in amplitude of the late volley. This facilitation suggests that fibres directly activated by the stimulation pulse excited the RST synaptically. An acute lesion to the DLF contralateral to the stimulation site within the RN eliminated the late descending volley and greatly reduced the synaptic potentials seen in the contralateral spinal cord. In this particular lesion experiment (see figure 2.8) the early volley was reduced by about 55%. In two experiments, only an early volley was seen in the contralateral spinal cord, which produced synaptic potentials in the intermediate grey of the contralateral only (R506) and the contralateral and ipsilateral spinal cord (R438). From the results obtained in the control animals, it can be concluded that the late volley represents synaptic activation of the RST. The origin of the early volley is more complicated. In some cases the early volley is probably produced by direct activation of the RST. However, in other cases where synaptic potentials are produced that do not correlate with the anatomy of the RST such as that shown in R478, the early

volley probably represents axons of others systems. It could be possible that the early volley represents more than one pathway, such as the RST and another pathway which could explain why the early volley is still present after a lesion to the DLF but is reduced in amplitude.

2.4 Discussion

The aim of this study was to assess the electrophysiological actions of the rubrospinal tract (RST) in control animals and in those with a lesion to the dorsal columns. The rubrospinal tract of the rat is often used in experimental models of spinal cord injury in which following an injury, changes in this tract are assessed as to whether they represent regeneration or plasticity. Electrophysiological studies are crucial in assessing functional reconnection of fibres following injury. For this, an understanding of the electrophysiological distribution of rubrospinal actions is required. This study was aimed at providing this crucial information, and once it was obtained, was used as a basis for comparison of possible changes within this tract following a cervical spinal cord injury (see next section).

2.4.1 Cord dorsum potentials

A tungsten electrode was located in the Red nucleus (RN) following stereotaxic coordinates and its location was confirmed by the recording of antidromic field potentials in the RN while stimulating the contralateral dorsolateral funiculus (DLF). Stimulation of the magnocellular region of the red nucleus (RNm) produced two descending volleys in the contralateral spinal cord and one in the ipsilateral spinal cord. Contralaterally, the evidence suggests that the early volley sometimes represents direct activation of the RST, but may involve other pathways other times,

while the late volley is almost certainly mediated by the RST (see later). Synaptic potentials were seen mainly in the contralateral spinal cord, but in two cases were also seen in the ipsilateral spinal cord. Because the evidence for the late volley being mediated by the RST is strong, this volley is discussed first, followed by discussion of the early volley. Discussion of observed field potentials will follow.

Stimulation of the RN produced two descending volleys in the contralateral DLF. The late descending volley was seen only in the contralateral DLF. This volley was usually larger when stimulating within the RNm and rostral to it, but not when stimulating caudal to the magnocellular region of the red nucleus. There are several lines of evidence that prove that this late volley is mediated by fibres from the rubrospinal tract. First of all, the RST is mainly a contralateral pathway that descends in the dorsolateral funiculus of the spinal cord, and this volley was seen only in the contralateral spinal cord within the DLF. Secondly, an acute lesion to the DLF ipsilateral to the recorded volley destroyed this volley and the fields associated with it (see later). And finally, in a ketamine and xylazine preparation, the late volley increased in amplitude with the number of stimulating pulses applied. This facilitation suggests that fibres directly activated by the stimulation pulse excite the RST synaptically.

It is known that rubrospinal cells receive major inputs from three different sources:

1. They receive monosynaptic excitatory and disynaptic inhibitory inputs from the frontal regions of the cerebral cortex (Tsukahara *et al.*, 1968).

2. From the cerebellum, RNm cells receive monosynaptic excitatory input transmitted via the contralateral interpositus nuclei through synapses that contact the somatic region of the RNm cells (Toyama *et al.*, 1968).

3. From the somatic periphery, the RNm cells receive excitatory and inhibitory influences through fibres ascending in the dorsal column system.(Padel & Jeneskog, 1981;Jeneskog & Padel, 1984).

Baldissera and colleagues (1972) demonstrated synaptic activation of the RST and provided evidence that this activation is via interpositorubral fibres. In the cat, they showed that stimulation of the RN evoked a two component discharge: the direct and synaptic (Baldissera *et al.*, 1972). Both components were abolished following a lesion to the RST at the level of the medulla. The synaptic component was thought to be caused by synaptic activation of rubrospinal neurons. Baldissera and colleagues reported that when stimulating ventral in the RN pre-synaptic interpositorubral fibres are activated leading to synaptic activation of rubrospinal neurons. They verified this by recording antidromic field potentials from the interpositus. Antidromic activation of axons from the ventral spinocerebellar tract may contribute to the descending discharge as they run close to the contralateral RN and would also be interrupted by the lesion to the medulla. But, Baldissera and colleagues ruled this out, as no evidence exists for collaterals from the spinocerebellar tract to the RN (Massion, 1967). In addition to this, during their experiments, they showed that discharge evoked from the RN was abolished following a lesion to the RST at the level of the spinal cord which did not interrupt the spinocerebellar tract. Synaptic activation via the corticospinal tract (CST) was also excluded. A lesion to the lateral brain stem destroying the RST but not the CST did not evoke a discharge in the contralateral dorsal quadrant of the spinal cord following stimulation of the RN using a strong stimulus current.

In the mouse, i*n vitro* monosynaptic responses in the RNm were elicited by an electrode array placed contralaterally near the midline. Activation of interpositorubral axons lead to synaptic excitation of the RNm (Jiang *et al.*, 2002). The electrodes

were arranged in a line with the most rostral end at the level of the rostral edge of the RNm, and the most caudal end was located at the level of or just caudal to the decussation of the cerebellar peduncle. It was found that the optimum location for synaptic activation of RNm neurons was from the electrode at the most caudal end, as this was the best site for activation of axons from the interpositus nucleus. In the current experiments, the synaptic volley was evoked from a number of sites within the RNm. It was largest when stimulating rostrally within the RNm. However, it was also seen when stimulating the parvocellular region of the RN (see R521 in table 1). Fibres of the interpositus are known to enter the RN at its caudomedial border, course rostrally through the entire RN and emerge laterally on their way to the thalamus (Caughell & Flumerfelt, 1977). Therefore a synaptic volley can be evoked from the parvocellular and magnocellular part of the RN (see also figure 2.4). With the known course of projection of the interpositus fibres, it seems strange that a synaptic volley was not seen in the ipsilateral spinal cord during an experiment in which the stimulation site was caudal to the RN, and close to the decussation of the superior cerebellar peduncle (see figure 2.4, AP1.96, 200μA). At this caudal location, it would have been expected here that axons of fibres from the interpositus would have been activated leading to synaptic excitation of RST fibres in the RN contralateral to the stimulus. But even at 200μA this didn't occur, which leads to this unexplained observation.

The above studies of Baldissera *et al* and Jiang *et al* did not investigate the possibility of synaptic activation of RN neurons via the medial lemniscus. The medial lemniscus conveys fibres of various origins:

1. Fibres ascending in the dorsal columns
2. Fibres ascending from the ventral quadrant of the spinal cord

Padel and colleagues provided evidence for the existence of an excitatory somatosensory pathway that runs through the dorsal columns and reached the RN after a relay in the dorsal column nuclei (Jeneskog & Padel, 1984).

It was later shown that a lesion to the dorsal columns in the most rostral segments of the spinal cord did not interrupt the somatosensory responses in the RN cells in a decorticate and decerebellate cat (Padel *et al.*, 1986). This provided evidence for a pathway with a relay at segmental levels. It was shown that that the primary afferents enter the dorsal columns and give off collaterals into the spinal grey and form synapses at the segmental levels. After one or more relays, these collaterals connect neurons whose axons ascend through the contralateral ventral quadrant of the spinal cord. Fibres reach the medial lemniscus and intermingle with fibres from the dorsal column nuclei. These fibres give off collaterals to the RN before they reach the thalamus (Padel & Relova, 1988). This pathway was identified as a spinorubral pathway to the RNm which was later shown to originate from spinothalamic fibres giving off collaterals at the level of the midbrain (Relova & Padel, 1989).

As for the connection between the RN and the medial lemniscus, a more recent study showed that stimulation of the medial lemniscus induced short-latency postsynaptic potentials in rubrospinal cells. Monosynaptic excitatory postsynaptic potentials, and disynaptic inhibitory postsynaptic potentials were evoked in RNm cells (Padel *et al.*, 1995). The studies by Padel and colleagues therefore provide evidence for a strong projection from the medial lemniscus to the RNm. The possibility therefore exists that synaptic excitation of RN neurons can occur through activation of fibres of the medial lemniscus.

The early volley was seen in both the ipsi- and contralateral DLF. This volley was seen in the ipsilateral DLF when stimulating more caudally within the RNm, and also

caudal to it. In the contralateral DLF, it was only seen when stimulating within the caudal RNm. In two out of seven experiments, the ipsilateral early volley produced synaptic potentials within the ipsilateral spinal cord. The fact that this volley had a similar conduction velocity to the late volley indicates that it may represent fibres of the same pathway, in this case the RST. The hypothesis here is that the early volley most often represents direct activation of the RST, as described by Baldissera (1972), where direct activation was evoked from the hindlimb region of the RN and also in the medial region where axons leave. Direct activation of the RST occurred only with stronger stimulus intensities when stimulating the ventral region of the RN (Baldissera *et al.*, 1972). However, in other cases, especially where the distribution of synaptic potentials does not correspond to the known anatomical projection pattern of the RST, the early volley may represent fibres of other systems. This would provide an explanation as to why following a lesion to the DLF the early volley did not disappear but was reduced in size (see figure 2.9). The possibility that the CST is involved in mediating this early volley is ruled out because the conduction velocity of the CST (about 30 ms^{-1}) is much slower than that observed here (Enríquez-Denton *et al.*, 2001;Enríquez-Denton *et al.*, 2002;Alstermark *et al.*, 2004). One of the systems that may contribute to this early volley is the spinorubral pathway in which fibres ascend from the ventral quadrant of the spinal cord and terminate in the RN (as described above). Depending on the location of the stimulation site, the ipsilateral early volley may represent axons from the RN contralateral to the stimulus site. This is usually seen following a caudal and ventral stimulus site within the RN, as axons from the RN contralateral to the stimulus can be activated as they cross. This would result in the presence of ipsilateral RST synaptic potentials (see later).

2.4.2 Intraspinal recordings

Stimulation of the RNm produced synaptic potentials in the contralateral cervical spinal cord (C5-C8). The descending volley was seen in the region of the DLF and the distribution of its amplitudes as inferred from the isopotential maps demonstrated a more medial distribution spreading towards the dorsal horn. Analysis of these potentials showed terminal like potentials in some of the traces. It is possible that the spread seen in the isopotential maps represents collaterals of rubrospinal fibres crossing into the grey matter. Intraspinal field potential maps were produced for seven animals, in which all showed a distribution of synaptic potentials within the intermediate grey of the contralateral spinal cord, although two were dominated by ventral synaptic fields. Most often, it was difficult to disclose whether the synaptic potentials were produced by the early or late volley due to these volleys displaying similar thresholds. In one particular example, due to a difference in threshold of the early and late volley, the synaptic fields were correlated with the late volley (see figure 2.8). Increasing the intensity of stimulation in the RNm produced an increase in the amplitude of the synaptic potentials which correlated with the increase in amplitude of the late volley.

An acute lesion to the DLF ipsilateral and rostral to the recorded fields was carried out in three animals. In the first animal, the destruction of the DLF resulted in the elimination of the late volley and the synaptic potentials were greatly reduced (Figure 2.9). In the second animal, with a similar lesion, both volleys and the synaptic fields disappeared. The third animal, in which the lesion destroyed the medial aspect of the DLF, both volleys were reduced by about 50% and the synaptic fields by about 80%. The ipsilateral volley in all three animals was unaffected. These observations provide evidence that the late volley and the synaptic potentials associated with it are mediated by rubrospinal fibres. The contralateral early volley, as previously described

here most often represents direct activation of the RST, but can also represent axons of other pathways.

It has previously been demonstrated in the cat that the signals carried by rubrospinal axons are conveyed through spinal interneurons to indirectly activate motoneurons. Electrophysiological (Hongo *et al.*, 1965;Hongo *et al.*, 1969;Antal *et al.*, 1992) and anatomical (Antal *et al.*, 1992) evidence showed that the RST is an excitatory pathway that establishes synaptic contacts on inhibitory and excitatory interneurons. These interneurons are connected to the RN via a monosynaptic pathway (for review see Massion 1967). This is in agreement with the distribution of synaptic potentials seen in the present study, in which synaptic potentials were seen mainly within the intermediate grey. It has been previously reported that rubrospinal fibres make close appositions to motoneurons (Kuchler *et al.*, 2002). However, only one animal with an intraspinal map at C8 (R533) showed a negative focus that spread towards the most dorsolateral motor column (lamina IX). In line with this, the anatomical observations of this thesis showed that RST fibres that project to the most dorsolateral lamina IX are more common at C8 than C7 (cf. anatomy chapter). However, it must be noted that the observation of terminals in the lamina IX anatomically or physiologically does not necessarily indicate that the RST fibres synapse directly onto motoneurons.

In three out of seven animals (R511, R438 & R478), synaptic potentials were seen within the ispilateral spinal cord. In animal R511, a large early volley was seen in the ipsilateral DLF, but only small synaptic potentials were recorded. The ventral and medial location of this animal's stimulation site could have resulted in the activation of fibres from the RN contralateral to the stimulation site, i.e., activation of its axons as they cross. However, it also cannot be ruled out that the early volley in R511 may represent fibres of other systems. The synaptic potentials of animal R438 (only an early volley in the CDP) were dominated by ventral potentials, seen bilaterally. Small

synaptic potentials were see in the intermediate grey of only the contralateral spinal cord. It seems likely that the early volley of this animal represents another pathway in addition to that of the RST. The contralateral synaptic potentials within the intermediate region may well be mediated by the RST, but the more ventral bilateral potentials are probably due to the activation of fibres from a different pathway.

The intraspinal map of animal R478 was dominated by a bilateral ventral and medial distribution of synaptic potentials, with only small synaptic potentials seen in the intermediate grey of the contralateral spinal cord. The distribution pattern of the isopotentials map is not what has commonly been seen in the current studies. In animal R478 and by observing individual sweeps, firing of units were seen which may represent motoneurons firing. The ventral synaptic potentials observed may therefore represent actions of the reticulospinal pathways as it is known that the mesencephalic reticular formation projects to the ipsilateral spinal cord (Holstege & Cowie, 1989;Holstege, 1991).

2.4.3 Conclusion

Stimulation of the RN most often produced two volleys in the caudal cervical spinal cord of the rat. The late volley represents synaptic activation of RN neurons. These results are in agreement of those reported in the cat. The contralateral early volley most often represents direct activation of the RST, but in some cases may involve pathways of other systems. The ipsilateral early volley may sometimes represent activation of axons from the RN contralateral to the stimulus site. A synaptic volley can be evoked in most regions of the RN due to the activation of fibres of the interpositus which are known to course through the whole RN. Stimulation at sites caudal to the RN can also lead to direct activation of RST fibres, especially if the stimulus site is close to the RST fibres that originate from the RN contralateral to the

stimulus site. Also stimulation caudal to the RN can evoke a synaptic response if the stimulus site is close to the decussation of the superior cerebellar peduncle (this is illustrated more in the next section). Therefore, stimulation of the RN does not always result in exclusively contralateral synaptic potentials. The CDPs and synaptic potentials seen are dependent on the final location of the stimulation site within or near the RN. The common pattern of the distribution of synaptic potentials is usually seen in the contralateral intermediate zone. Only one animal with an intraspinal map recorded at C8 (R533) showed synaptic potentials which spread to the most dorsolateral lamina IX.

2.5 Results- Lesioned animals

The results of seven lesioned animals are reported here. All animals received a C4/C5 lesion to the dorsal columns, followed by behavioural testing and finally by a terminal electrophysiological experiment. The behavioural assessments included three tests, the pellet retrieval test, the sticker removal test, and the cylinder test out of which the pellet retrieval test proved to be the most discriminatory. Histological reconstructions of the lesion site showed that lesion extent to the dorsal columns varied between the animals and as a consequence the functional recovery of these animals also varied. Following reconstruction of the lesion sites, it was shown that two animals out of the seven (R538 & R574) sustained a lesion specific to the dorsal columns with no damage to the DLF and two animals sustained an incomplete lesion to the dorsal columns (R647 & R601). These animals recovered to pre-lesion success rates. Three animals sustained a lesion to the dorsal columns that extended to the left and/or right DLF and/or the ventral white matter R543, R539, & R590). These animals did not recover to pre-lesion success rates (see chapter 2). Once all

behavioural testing was complete, animals were allowed to recover for two weeks before the terminal electrophysiological experiment. The terminal experiment was carried out 14 weeks after the lesion was made (see chapter 2 for more details on behavioural assessments and results).

2.5.1 Cord dorsum potentials

The general result was that the electrophysiological actions of the RST in animals with a lesion to the dorsal columns were similar to that of control animals. The final stimulation site in the midbrain was confirmed by observing antidromic field potentials and in some experiments the location of the electrode was adjusted to where a large late volley was seen.

As in the control animals, stimulation of the red nucleus and surrounding areas produced two volleys in the cervical spinal cord of the rat. Table 3 shows data taken from a number of lesioned animals in which the volleys observed in the contra- and ipsilateral CDP from the cervical spinal cord are illustrated. The stimulation site in the midbrain is shown. However, in those animals with a stimulation site caudal to the RN (600μm caudal), a picture from the rat atlas is used in place of a photomicrograph. No illustration is shown for animal R538, as a corresponding picture from the rat atlas is not available. The distance from the caudal edge of the RN is stated in each animal. As in the controls, stimulation of the caudal part of the RN produced an early volley in both the contra- and ipsilateral spinal cord (R539 in table 3). The ipsilateral early volley was mostly larger in amplitude when stimulating caudal to the RN. When stimulating caudal to the RN, a late volley was seen in the contralateral spinal cord and an early one was seen in the ipsilateral spinal cord (R543-R538 in table 3). A late volley was seen in the CDP of the ipsilateral spinal

cord of animal R547 (see table 3). The most rostral location for the stimulation electrode was found to be 1000μm from the caudal edge of the RN. The data shown in the table is ordered starting with those with the most caudal stimulation site.

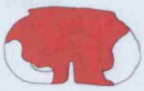
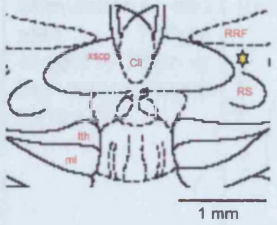
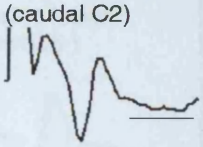
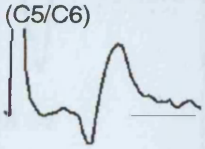
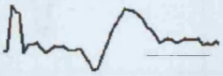
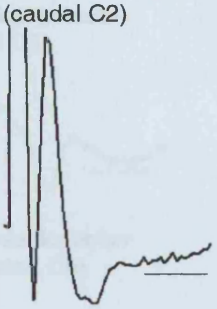
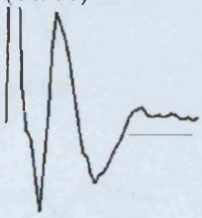
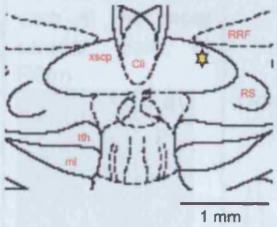
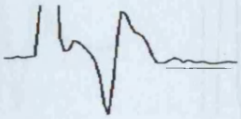
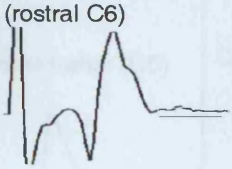
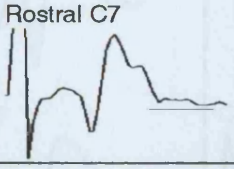
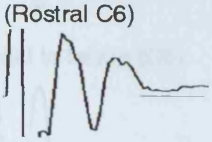
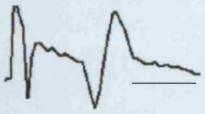
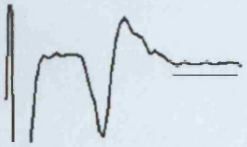
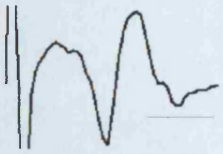
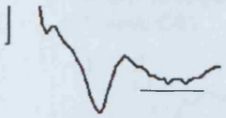
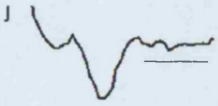
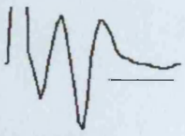
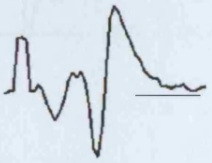
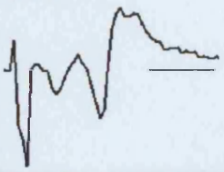
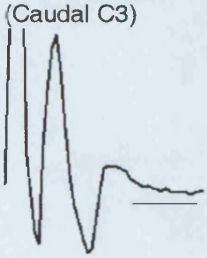
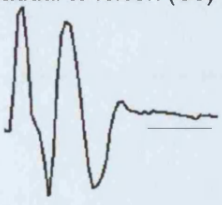
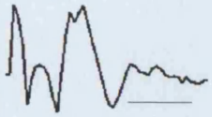
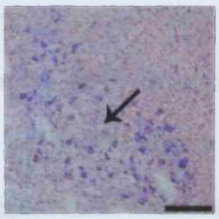
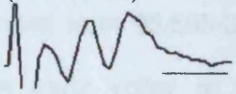
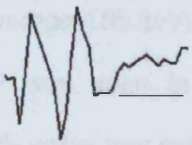
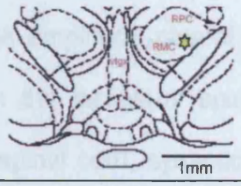
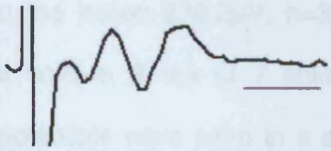
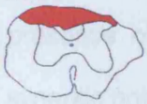
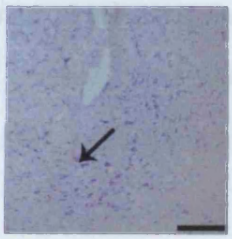
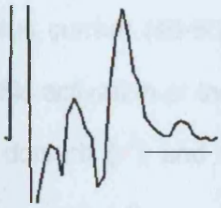
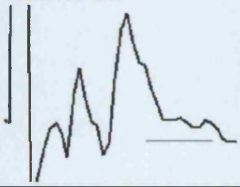
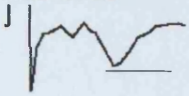
Experiment	Stimulation site- Distance from caudal edge of RN of (μm)	Contra CDP	Ipsi CDP
R543 	Stimulation site was 600μm caudal to RNm 	50μA Rostral to lesion (caudal C2)  Caudal to lesion (C5/C6)  C7/C8 	50μA Rostral to lesion (caudal C2)  Caudal to lesion (C5/C6)  C7/C8 
R547 	Stimulation site was 600μm caudal to RNm 	50μA Rostral to lesion (caudal C3)  Caudal to lesion (rostral C6)  Rostral C7 	50μA Caudal to lesion (Rostral C6) 

Table 2.3 Summary of stimulation sites within the midbrain and their corresponding cord dorsum volleys. Photomicrographs show the lesion indicating the stimulation site in the RN and the distance from caudal edge of the RN is stated. – indicates rostral + indicates caudal. Results appear in a caudal to rostral order. Scale bar in traces is 1ms, and in micrographs 200μm.

Experiment	Stimulation site- Distance from caudal edge of RN of (μm)	Contra CDP	Ipsi CDP
R538 	Stimulations site was 100μm caudal to RNm at the correct depth for the RN	50μA Rostral to lesion (Rostral C3)  Caudal to lesion (Rostral C5)  Caudal C6 	50μA Rostral to lesion (Mid C3)  Caudal to lesion (Rostral C5)  Caudal C6 
R539 	Stimulation site was at the most caudal pole of RNm 	40μA Rostral to lesion (Caudal C3)  Caudal to lesion (C5)  C6/C7 	40μA Rostral to lesion (Caudal C3)  Caudal to leison (C5)  C6/C7 

Experiment	Stimulation site- Distance from caudal edge of RN of (μm)	Contra CDP	Ipsi CDP
R574 	-300 / Mid RNm 	50μA Caudal to lesion (Rostral C6) 	50μA Caudal to lesion (Rostral C6) 
R590 Error! Objects cannot be created from editing field codes.	-650 / through the lateral aspect of the RN 	50μA Caudal to lesion (Mid C6) 	Data missing
R601 	-1000 / slightly medial and ventral of RNm 	50μA Caudal to lesion (rostral C6)  Rostral C8 	50μA Caudal to lesion (Rostral C6)  Rostral C8 

2.5.2 Intraspinal recordings

Isopotential maps were produced in seven animals. Out of these seven, a late volley corresponding to the late volley in the CDP was seen in the contralateral spinal cord, within the dorsolateral funiculus of all seven animals. The maximum amplitude for the late volley caudal to the lesion ranged from 95.605-378μV (average 185.3μV). An early volley corresponding to the early volley in the CDP was seen in the contralateral spinal cord in 2 out of the 4 animals in which an early volley was seen in the CDP (average amplitude caudal to the lesion 218μV), and was seen in the ipsilateral spinal cord in all 6 of the animals in which an early volley was seen in the CDP (average amplitude caudal to the lesion 239.2μV, n=3). Synaptic potentials were seen in the ipsilateral spinal cord in 3 out of 7 animals (table 4). In the contralateral spinal cord, synaptic potentials were seen in a similar location to that seen in the control animals. The maximum amplitude ranged from 71-258μV. Table 4 shows a summary of the presence of the volleys in the cord dorsum and in the intraspinal recordings. A low stimulus current (40-60μA) was used in the midbrain with the intention of achieving specific activation of the RST. The ticks represent the presence of a volley in the cord dorsum (✓) and the presence of a volley and synaptic potentials in the intraspinal records (✓).

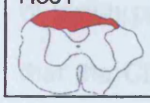
	Contra			*Ipsi*		
	Early volley	Late volley	Synaptic potentials	Early volley	Late volley	Fields
R538 		✓ ✓	✓	✓ ✓		not sampled
R539 	✓	✓ ✓	✓	✓ ✓		✓
R543 		✓ ✓	✓	✓ ✓		✓
R547 		✓ ✓	✓	✓ ✓		✓
R574 	✓ ✓	✓ ✓	✓	✓ ✓		not sampled
R590 **Error! Objects cannot be created from editing field codes.**	✓ ✓	✓ ✓	✓	✓ ✓		
R601 	✓	✓ ✓	✓			

Table 2.4 ummary of cord dorsum and intraspinal potentials in lesioned animals.
Stimulation of the red nucleus often produced two descending volleys (early and late) in the contralateral spinal cord and one volley in the ipsilateral spinal cord (early). ✓ Refers to cord dorsum potentials caudal to the lesion ✓ Refers to intraspinal potentials caudal to the lesion.

Due to the variation in the location of the stimulation site in the midbrain, field potentials for four animals with a stimulation site within the red nucleus are described first (R601, R590, R574, & R539). In the following descriptions, unless otherwise stated, the volleys referred to are those seen in the intraspinal records.

Intraspinal recordings in R601 were made at C7 and histological confirmation of the stimulation site showed that this was within the centre of the parvicellular region of

the RN, 1000μm rostral to the caudal edge of the RN. Reconstruction of the lesion showed that it spared the ventral part of the dorsal columns, which is where the main crossed component of the CST is located. A late volley and synaptic potentials were seen in the contralateral spinal cord (Figure 2.18). No volley or synaptic potentials were seen in the ipsilateral spinal cord.

For R590, intraspinal recordings were made at C7 and histological confirmation of the stimulation site showed that it was within the centre of the RNm, 650μm rostral to the caudal edge of the RN. Reconstruction of the lesion showed that it completely destroyed the dorsal columns and also extended to the ipsi- and contralateral DLF. Although the recording of both the antidromic and the CDPs shows that the RST is largely surviving. In the contralateral spinal cord, two volleys were seen, the late one and the early one. Preceding the focus of negativity for the late volley, a positive focus was seen. This may be an indication of damage to fibres due to the extension of lesion pathology to the contralateral DLF. Taking this into account, and the fact that the CDP in the contralateral spinal cord looks fine, it is clear that some RST fibres were damaged by the lesion, while others survived. A very small early volley (30μV) was seen within the ipsilateral spinal cord in the DLF. This volley was seen only in the very last track of the map (1-1.5mm deep). Further tracks more lateral were not sampled and therefore a focus for this volley could not be determined. Synaptic potentials were seen in the intermediate grey of the contralateral spinal cord (Figure 2.19).

Animal R574 sustained a complete lesion to the dorsal columns with no extension to the DLF or the ventral spinal cord. Intraspinal recordings were made at C7 and histological confirmation of the stimulation site showed that it was at the centre of RNm 350 μm rostral to the caudal edge of the RN. Two volleys, an early and a late one were seen in the intraspinal recordings in the contralateral cord. These volleys

appeared within the same location in the contralateral DLF. Synaptic potentials were seen in the contralateral spinal cord within the intermediate grey (Figure 2.20). Full tracking of the ipsilateral spinal cord was not carried out in this animal; therefore, no map was plotted for the ipsilateral spinal cord. However, an early volley was seen in the ventral part of the ipsilateral spinal cord (Figure 2.21) but no synaptic potentials were seen. This volley had a focus within the ventral quadrant of the ipsilateral spinal cord and was at its maximum (115μV) at 1.1mm from the midline and at a depth of 1.9mm (not shown in map, see figure 2.21).

The last animal out of this group is R539. An isopotential map was produced for this animal at C7 and histological confirmation of the stimulation site within the midbrain showed that it was at the most caudal edge of the RN. Post mortem examination of the lesion showed that it completely destroyed the dorsal columns and also extended slightly to the contra- and ipsilateral DLF. However, the volleys in the CDP seem normal and it seems here that although anatomically the DLFs looked damaged, physiologically the RST seems to be functional. A late volley was seen within the DLF of the contralateral spinal cord (Figure 2.22). Synaptic potentials were seen within the intermediate grey of the contra- and ipsilateral spinal cord. In this animal, RST fibres from the RN contralateral to the stimulus site could have been activated here resulting in the ipsilateral synaptic potentials.

Figures 2.18-2.20 & 2.22-2.25 Field potential maps for animals with a cervical spinal cord lesion. The electrophysiological actions of the RST in the caudal cervical spinal cord of lesioned animals are illustrated in isopotential maps. Photomicrographs of the final stimulation sites in the midbrain (MB) are shown. In those were a photomicrograph could not be produced, a picture taken from Paxinos' atlas is used to illustrate the stimulation site. The antidromic field potentials at the stimulation site are also shown except for R601 (final position for the electrode chosen to give a late volley), R538, R543, and R547 where the stimulation site for these animals was caudal to the RN.The descending volleys and synaptic potentials are illustrated. The latencies at which the amplitudes for these potentials were measured are indicated by the dotted lines. The same calibration bar (-400 to +400μV) is used for all figures except for R533, R536, and R601.

Figure 2.18 R601

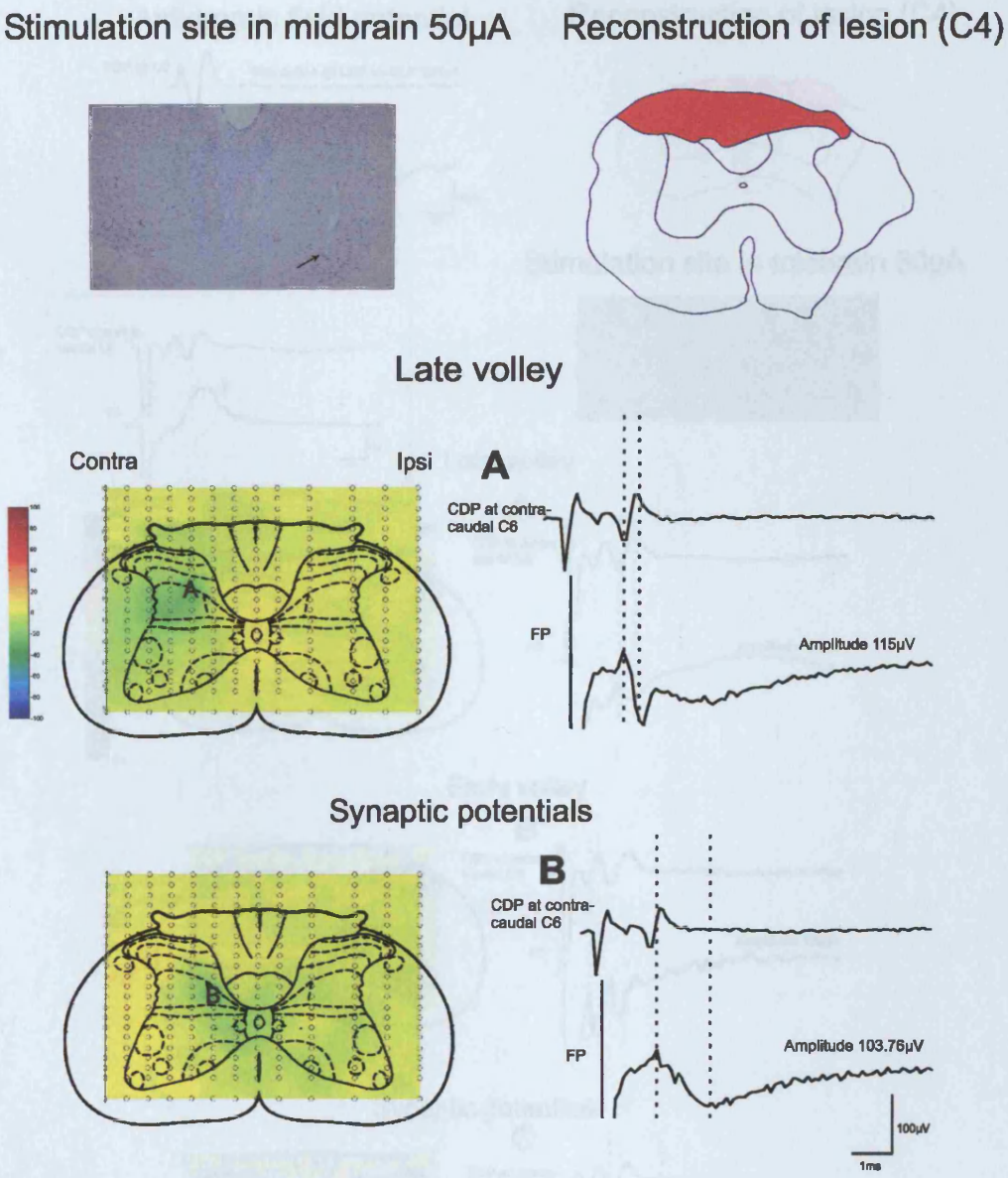


Figure 2.19 R590

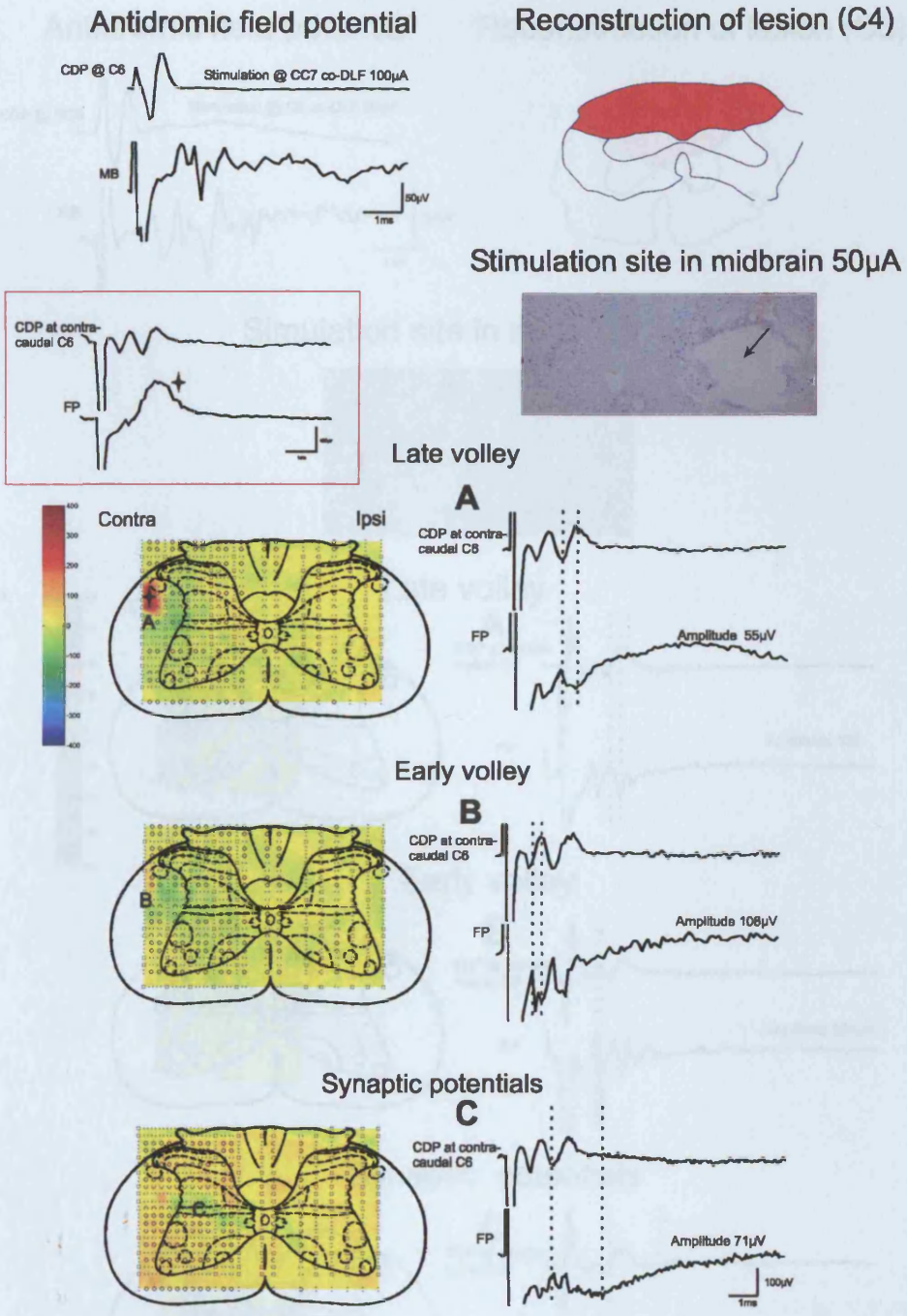
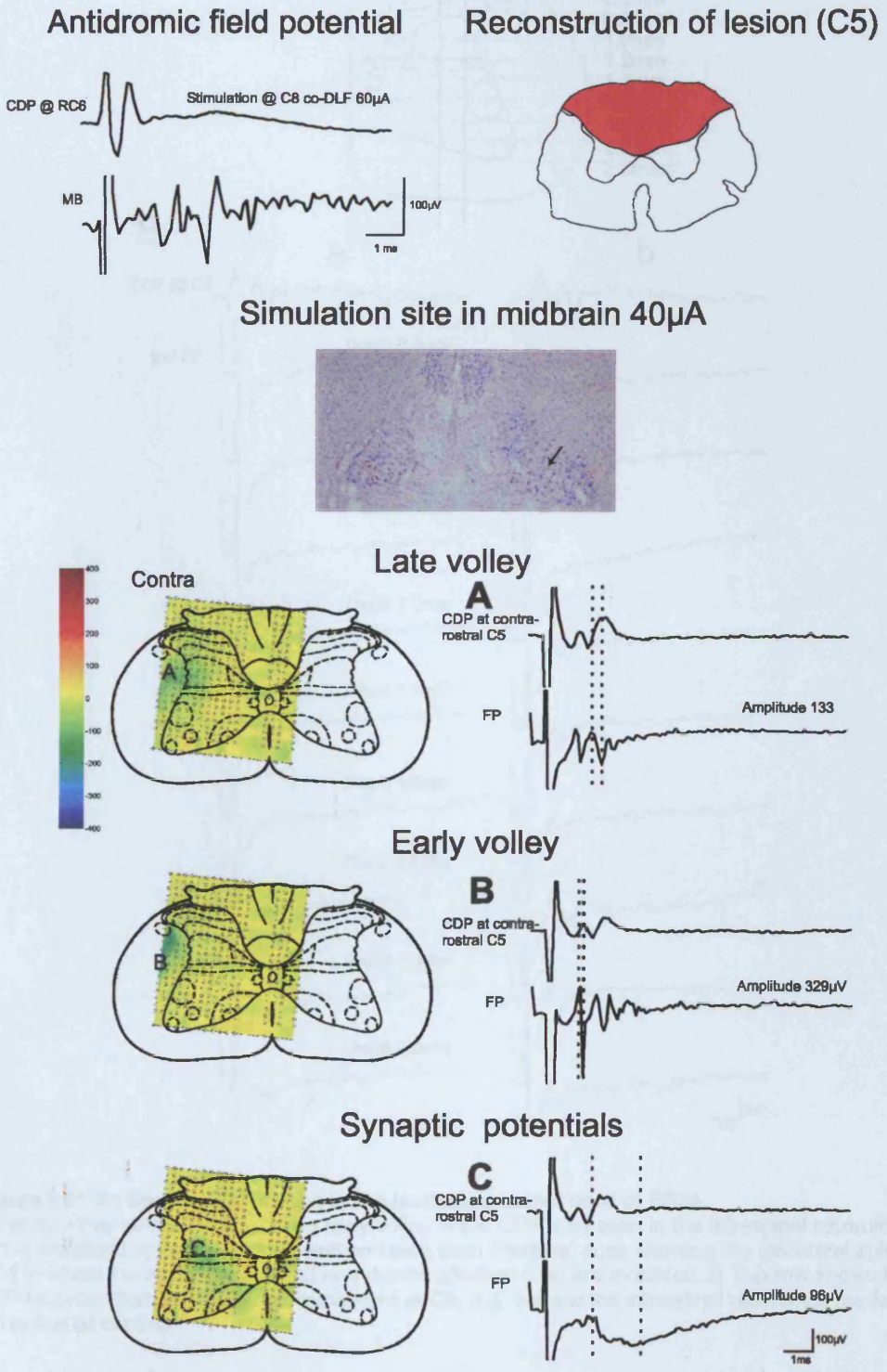


Figure 2.20 R574



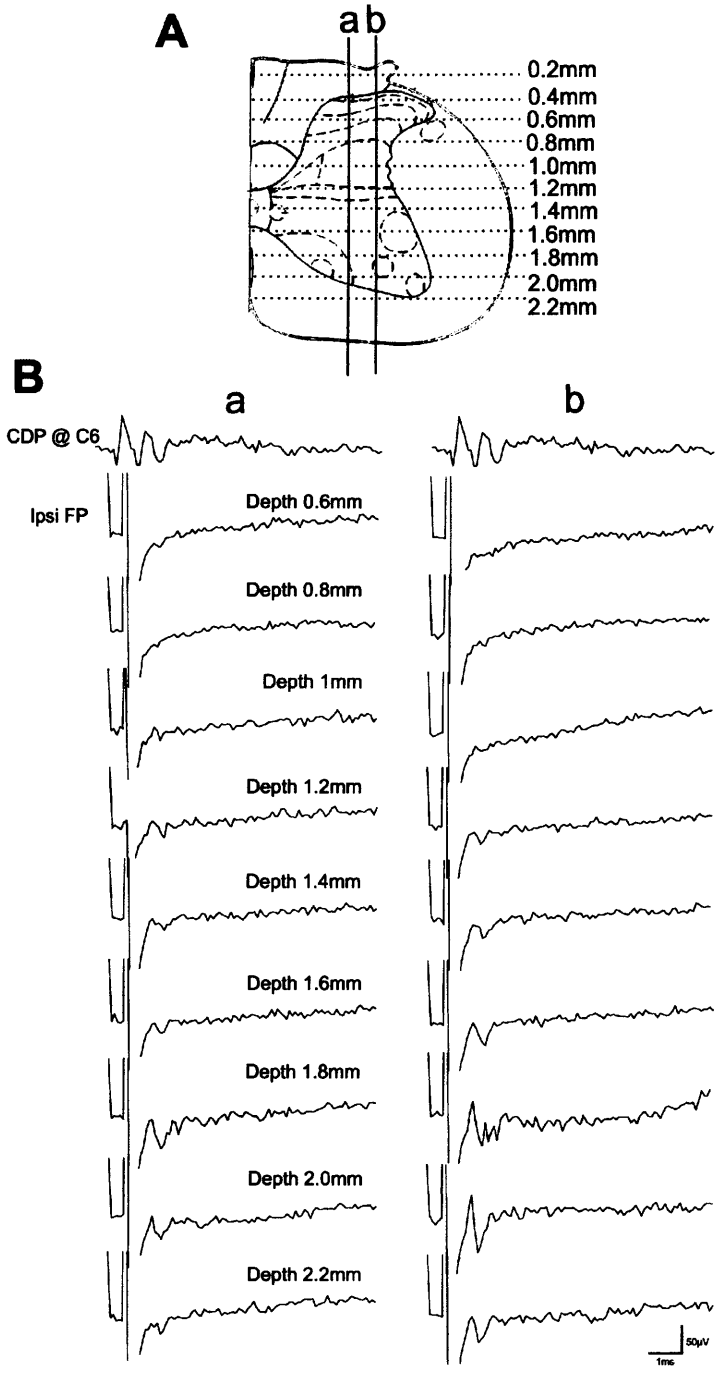
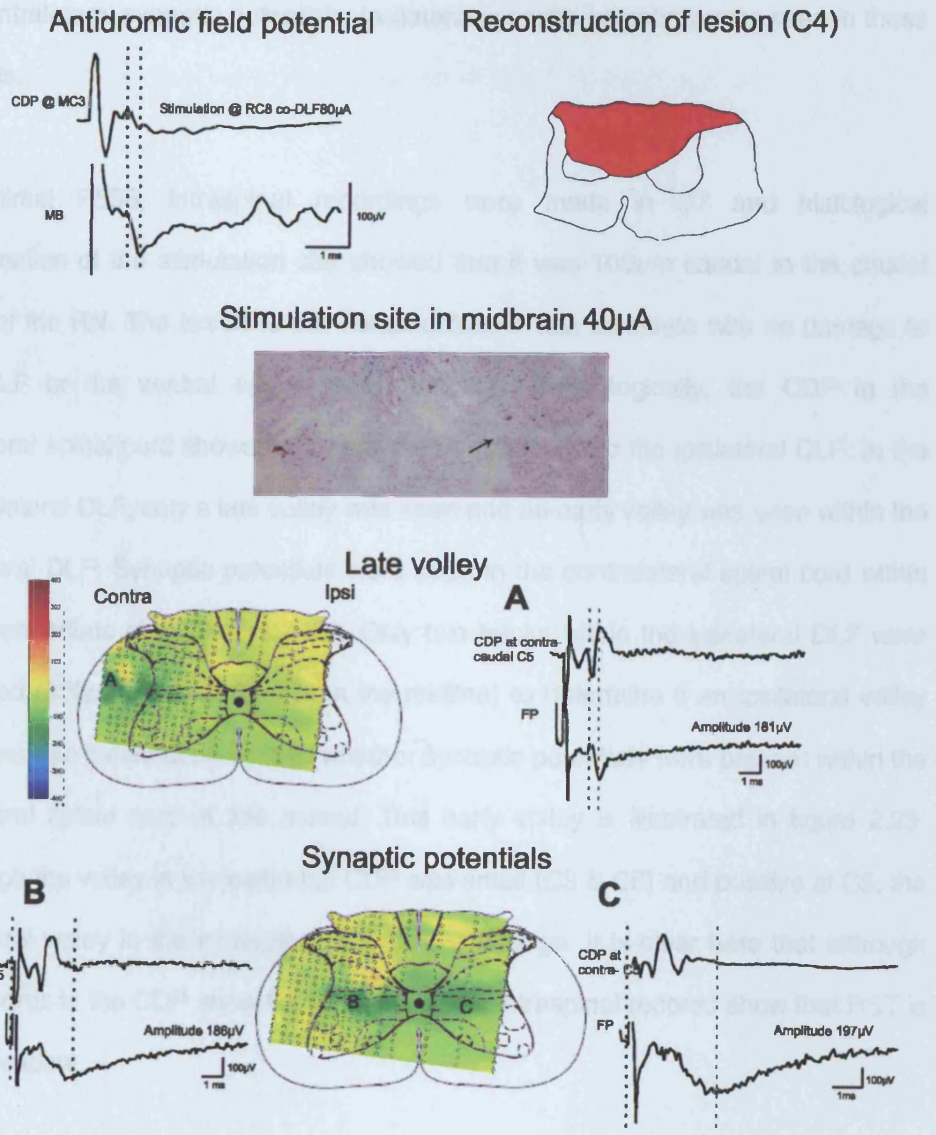


Figure 2.21 An Early volley is seen in the ipsilateral spinal cord of R574.
An early volley corresponding to the early volley in the CDP was seen in the intraspinal recordings of the ipsilateral spinal cord. A Illustration taken from Paxinos' atlas showing the ipsilateral spinal cord in which the tracks (solid lines) and depths (dashed line) are indicated. B Top row shows the CDP recorded from the ipsilateral spinal cord at C6. *a* & *b* show the intraspinal recordings made at the indicated depths.

Figure 2.22 R539



The next group of animals (R538, R543, & R547) to be described are those in which the location of the stimulation electrode was found caudal to the RN. In addition to the contralateral synaptic potentials, ipsilateral synaptic potentials were seen in these animals.

In animal R538, intraspinal recordings were made in C7 and histological confirmation of the stimulation site showed that it was 100μm caudal to the caudal edge of the RN. The lesion to the dorsal columns was complete with no damage to the DLF or the ventral spinal cord. Although, physiologically, the CDP in the ipsilateral spinal cord shows that there may be damage to the ipsilateral DLF. In the contralateral DLF, only a late volley was seen and an early volley was seen within the ipsilateral DLF. Synaptic potentials were seen in the contralateral spinal cord within the intermediate grey (Figure 2.23). Only two tracks within the ipsilateral DLF were sampled (900μm and 1300μm from the midline) to determine if an ipsilateral volley was seen, so it cannot be verified whether synaptic potentials were present within the ipsilateral spinal cord of this animal. This early volley is illustrated in figure 2.23. Although the volley in the ipsilateral CDP was small (C3 & C6) and positive at C5, the ipsilateral volley in the intraspinal recording was large. It is clear here that although the records in the CDP show some damage, the intraspinal records show that RST is still functional.

For animal R543, intraspinal recordings were made at C6. The lesion to this animal was the most severe out of all lesioned animals. The lesion extended beyond the dorsal columns into both the contra- and ipsilateral DLF. Lesion pathology also extended ventral to the central canal, damaging the ventral CST and the medial part of the ventral quadrant. Histological confirmation of the stimulation site showed that it was 600μm caudal to the caudal edge of the RN. The late volley was seen within the contralateral DLF. Synaptic potentials were seen within the intermediate grey of the

contra- and ipsilateral spinal cord (Figure 2.24). An early volley was seen only in the ipsilateral spinal cord. The latency of the ipsilateral synaptic potentials was slightly earlier than that of the contralateral ones. Amplitude measurements for these potentials were measured at the indicated dotted line as shown on the traces in figure 2.24.

The final animal of this group is R547. The lesion to the animal was incomplete sparing a small amount of matter in the ventral aspect of the dorsal columns and therefore sparing some of the CST. Intraspinal recordings were made at C6 and histological confirmation of the stimulation site demonstrated that it was 600μm caudal to the caudal edge of the RN. A late volley was seen in the contra- and ipsilateral DLF (see also table 3). An early volley was seen in the ipsilateral spinal cord in the DLF. Synaptic potentials were seen in the intermediate grey in both the ipsi- and contralateral spinal cord (figure 2.25). The latency of onset and peak of the synaptic potentials were very similar in both sides of the cord.

Figure 2.23 R538

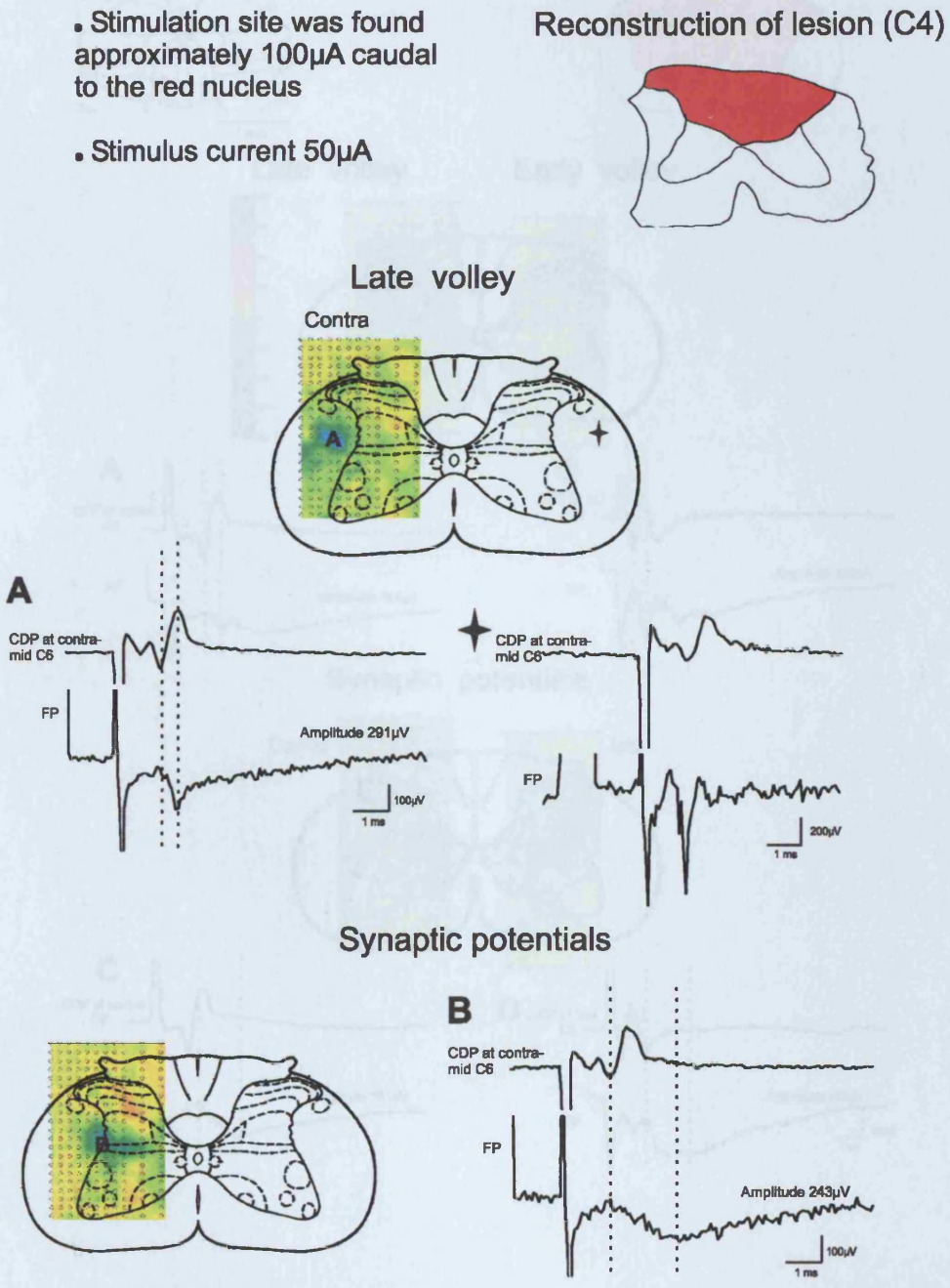


Figure 2.24 R543

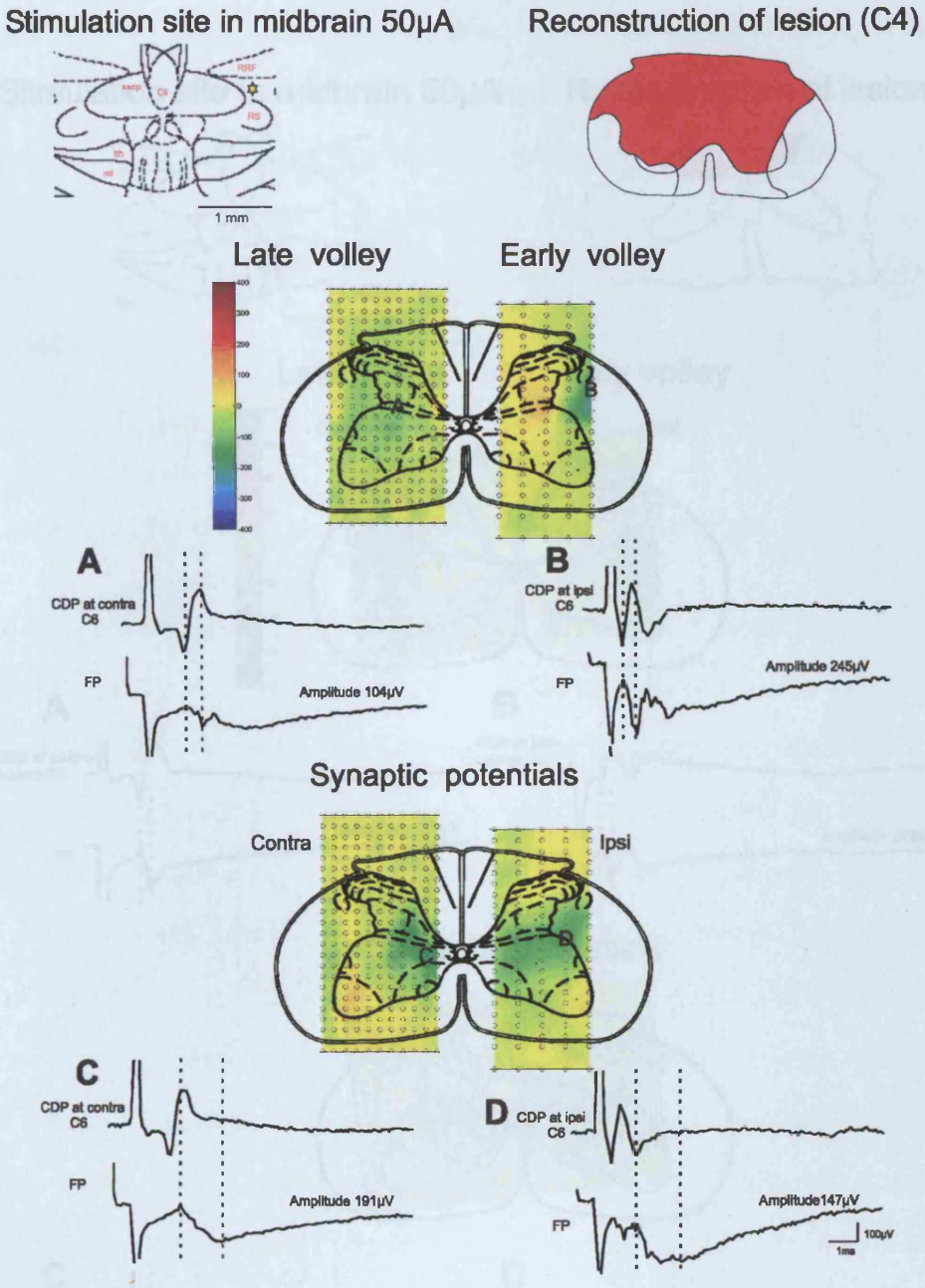
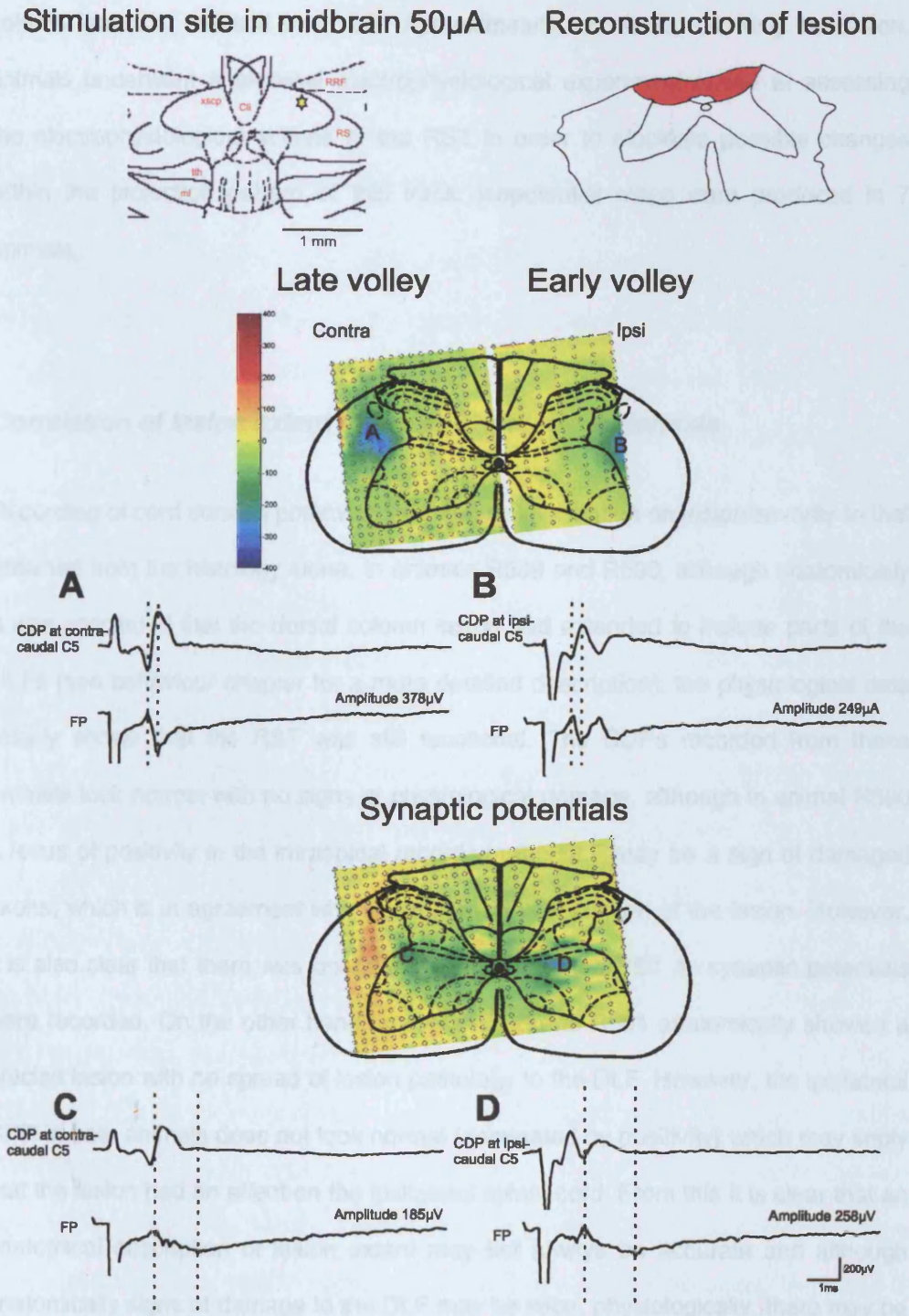


Figure 2.25 R547



2.6 Discussion- Lesioned animals

Following behavioural training (see behaviour chapter) animals underwent a dorsal column lesion at cervical level 4/5. Approximately 11 weeks following the lesion, animals underwent a terminal electrophysiological experiment aimed at assessing the electrophysiological actions of the RST in order to elucidate possible changes within the projection pattern of this track. Isopotential maps were produced in 7 animals.

Correlation of lesion extent to the cord dorsum potentials

Recording of cord dorsum potentials added new information on lesion severity to that obtained from the histology alone. In animals R539 and R590, although anatomically it was concluded that the dorsal column lesion had extended to include parts of the DLFs (see behaviour chapter for a more detailed description), the physiological data clearly shows that the RST was still functional. The CDPs recorded from these animals look normal with no signs of physiological damage, although in animal R590 a focus of positivity in the intraspinal records in the DLF may be a sign of damaged axons, which is in agreement with the anatomical description of the lesion. However, it is also clear that there was only partial damage to the RST as synaptic potentials were recorded. On the other hand, animals R538 and R601 anatomically showed a precise lesion with no spread of lesion pathology to the DLF. However, the ipsilateral CDP in both animals does not look normal (dominated by positivity) which may imply that the lesion had an effect on the ipsilateral spinal cord. From this it is clear that an anatomical description of lesion extent may not always be accurate and although anatomically signs of damage to the DLF may be seen, physiologically, there may be partial preservation of the RST. In line with this, anatomically the DLF may seem

spared but physiologically RST fibres may be affected by the lesion. These observations here will have an implication in behavioural studies in which recovery is correlated with specific pathways within the spinal cord following a lesion (see behaviour discussion).

Cord dorsum potentials and intraspinal records

The general distribution of synaptic field potentials was similar to those described in the control animals. As in the control animals, the distribution of amplitudes for the volley sometimes spread medially towards the grey matter, and may represent collaterals of the RST which were represented by terminal like potentials seen within the recorded field potentials. Three out of seven animals (R543, R547, & R538) showed synaptic potentials within the ipsilateral spinal cord within the intermediate grey which may represent activation of RST fibres from the RN contralateral to the stimulus site due to the caudal location of the stimulation site (as discussed in control animals). In those animals with a stimulation site 600μm caudal to the caudal pole of the RN (R543 & R547) and by reference with the rat atlas, the stimulation site was close to the already crossed RST and close to the decussation of the superior cerebellar peduncle. Activation of the fibres of the interpositus could explain the presence of a contralateral late volley representing synaptic activation of the RST via the interpositus. The ipsilateral early volley most likely represents direct activation of RST axons from the RN contralateral to the stimulation site. The largest early ipsilateral cord dorsum volley was seen in animal R543 which is explained by the close proximity of the stimulation electrode to the RST (see table 3). A late volley was seen in the CDP of the ipsilateral spinal cord of animal R547. It is known that fibres from the interpositus course within the superior cerebellar peduncle and ascend rostrally to the RN after decussating at the level of the inferior colliculus (Caughell &

Flumerfelt, 1977). It could be possible that stimulation at this level caudal to the RN, and close to the decussation of the superior cerebellar peduncle activates ascending fibres from the interpositus which terminate in the RN contralateral to the stimulation site. This could explain the late volley seen in the ipsilateral CDP and intraspinal records of R547. It also cannot be excluded that this late ipsilateral volley may have contributed to the synaptic potentials seen in the ipsilateral spinal cord of this animal.

In animal R543, an early volley in the contralateral CDP was recorded rostral to the lesion but disappeared caudal to the lesion. Consistent with this, the early contralateral volley in animal R539 reduced in size caudal to the lesion when compared with the CDP rostral to the lesion. However, the synaptic volley remained the same. Therefore, these two animals provide further evidence that the early volley may not always be mediated exclusively by the RST. A possibility is that the early volley here represents a pathway which is affected following a lesion or only projects to rostral cervical segments. As discussed in control animals, one hypothesis is that the spinorubral tract may contribute to the early volley. For those animals with stimulation sites caudal to the RN, fibres from the tectospinal tract should be considered as a possible pathway that may contribute to the early volley. The tectospinal tract is known to originate from the neurons in the superior colliculus and projects mainly to the upper segments of the cervical spinal cord. Very few axons project to the cervical enlargement and none project past the first thoracic segment (Rose *et al.*, 1991). If these fibres do represent part of the early volley, it would explain the reduction in size caudal to the lesion (R539 & R543).

As to the question whether changes had occurred in the RST following a lesion to the CST, it is difficult to assess here due to the varied location of the stimulation site in the RN. However, even by comparing the animals from the lesioned group (R601, R590, R574, and R539) with a similar stimulation site to those of the controls, there

does not appear to be a difference in the projection pattern. The intense projection pattern seen in R543 and R547 can be explained by the close proximity of the stimulation electrode to the already crossed RST (see stimulus location in table 3).Therefore, there is no obvious evidence of plasticity in the current study.

Raineteau and colleagues reported that the RST does in fact reorganise following a bilateral lesion to the corticospinal tract, but is only significant following therapeutic intervention (Raineteau *et al.*, 2001). In their study, the CST was lesioned at the level of the medulla oblongata and sprouting of the RST was assessed. In lesioned animals treated with mAb IN-1, the number of collaterals emerging from RST increased 2-fold as compared to un-lesioned animals. There was no significant increase in the number of collaterals in lesioned non-treated animals. In a later study they showed that following the CST lesion and treatment with mAb IN-1, RST collaterals invaded the ventral horn but not the dorsal horn of the cervical spinal cord. Microstimulation of the RST confirmed this reorganisation (Raineteau *et al.*, 2002). Therefore, it can be hypothesised that significant sprouting of the RST following a lesion to the CST may only be seen following therapeutic intervention.

The possibility exists that the technique used here to record field potentials is not sensitive enough to disclose sprouting of a small number of fibres in un-treated animals. However, a study investigating whether the RNm contributed to compensation for motor impairments following a lesion to the CST at the level of the pyramids (unilateral) in the rhesus monkey showed a clear pattern of post-lesion reorganisation of RN mediated output effects in forearm muscles (Belhaj-Saif & Cheney, 2000). The animal was trained for a reach and prehension test and during all phases of movement micro-stimuli were applied to the RN while recording EMG activity from the forearm. They showed that the strong extensor preference of RST was lost in favour of a more equal extensor/flexor distribution that matched that of the

CST. They also showed that the increase in facilitation of flexor muscles was associated with a large increase in the number of RNm neurons that co-facilitate flexors and extensors and a decrease in the number of RNm neurons that produce facilitation of extensors only. However, it must be noted that the current study assesses plasticity at the level of the spinal cord and cannot be compared to the above study.

The data provided here can act as a pilot study for further studies into plasticity within the RST following spinal cord injury in an untreated or treated group of animals. The protocols established here also provide an important tool for the assessment of regeneration and plasticity in other experimental models of spinal cord injury.

Chapter 3. Behavioural observations

3.1 Introduction

Development of spinal cord injury models in animals is vital for evaluating the pathology, physiology, and effective therapies. Following an injury to the spinal cord, functional recovery often occurs either spontaneously or following therapeutic intervention. To assess possible functional recovery following a spinal cord injury, specific behavioural tasks are crucial in the evaluation of the recovery. These tasks must be relevant to the specific injury and are especially important if the intervention is aimed at producing functional recovery (for review see (Muir & Webb, 2000). Behavioural tasks must be selected and developed to assess the deficits cause by the injury, whether it is motor or sensory.

Laboratory rodents are often used in experimental spinal cord injury and perform a wide range of behaviours. These behaviours can be selected and targeted for the assessment of recovery. Analysis of the impairments following an injury allows the experimenter to relate specific behavioural outcomes to specific regions of the spinal cord, therefore limiting the role to certain spinal cord pathways. Lesioning of specific spinal cord pathways, wherever possible, will allow identification of the function of the pathway. Rat behaviours that can be assessed include full body behaviours such as locomotion and specific forelimb or hindlimb behavioural assessments such as reaching, vertical exploration, and sticker removal.

In this chapter, behavioural outcomes of animals before and after a cervical spinal cord injury are reported and discussed.

3.2 Methods

Following a lesion to the dorsal columns, animals were assessed for functional recovery using three behavioural tests: the pellet retrieval test, the cylinder test, and the sticker removal test. Animals were housed individually and were maintained on a 12:12h light: dark cycle in the animal house of the Institute of Neurology and were fed ad libitum on a standard diet except during the training and testing periods. All animals were examined and weighed daily.

Twenty adult female Sprague Dawley rats weighing 200-230g at the beginning of the experiment were used (Harlan, UK, Ltd). Behavioural training and testing was performed during the light phase of the light/dark cycle.

3.2.1 Experimental time scale

Each animal was trained and tested for the pellet retrieval test. This consisted of one session pre-lesion and three sessions post-lesion (Figure 3.1). The sessions were as follows:

1. Pre-lesion: one 2 week session consisting of five days of training to allow the animal to learn the task, and five days of testing, animals were filmed on the last day of the testing session.
2. Post-lesion: three five day testing sessions, each session separated by two weeks, animals were filmed on the last day of each session.

Prior to each session, rats were food-deprived for 48 hours, and their weight was not allowed to drop more than 20%. To achieve this, they received a measured amount of food once a day (5-8g) during the afternoon and received water ad libitum.

Following all testing sessions, animals were given a period of recovery in order to regain their weight. This period was of 4 days prior to the lesion, and of 2 weeks after each post lesion testing session.

Please note that in the rest of this thesis, the pre-lesion testing session is abbreviated as pre, the first post-lesion testing session as P1, the second post lesion testing session as P2, and the third post lesion testing session as P3.

The cylinder and stickers tests did not require training, thus, were videotaped using a Sony digital video camera (DCR-PC120E, Sony Corp. Tokyo, Japan) along with the pellet retrieval test on the fifth day of each testing session. Frame by frame analysis at 33 frames per second was carried out using Adobe Premiere 6.5 software (Adobe systems incorporated).

At the end of the behavioural assessments, the animal underwent either a terminal electrophysiology experiment or a tract tracing procedure. All animals were perfused with fixative at the end of the experiments.

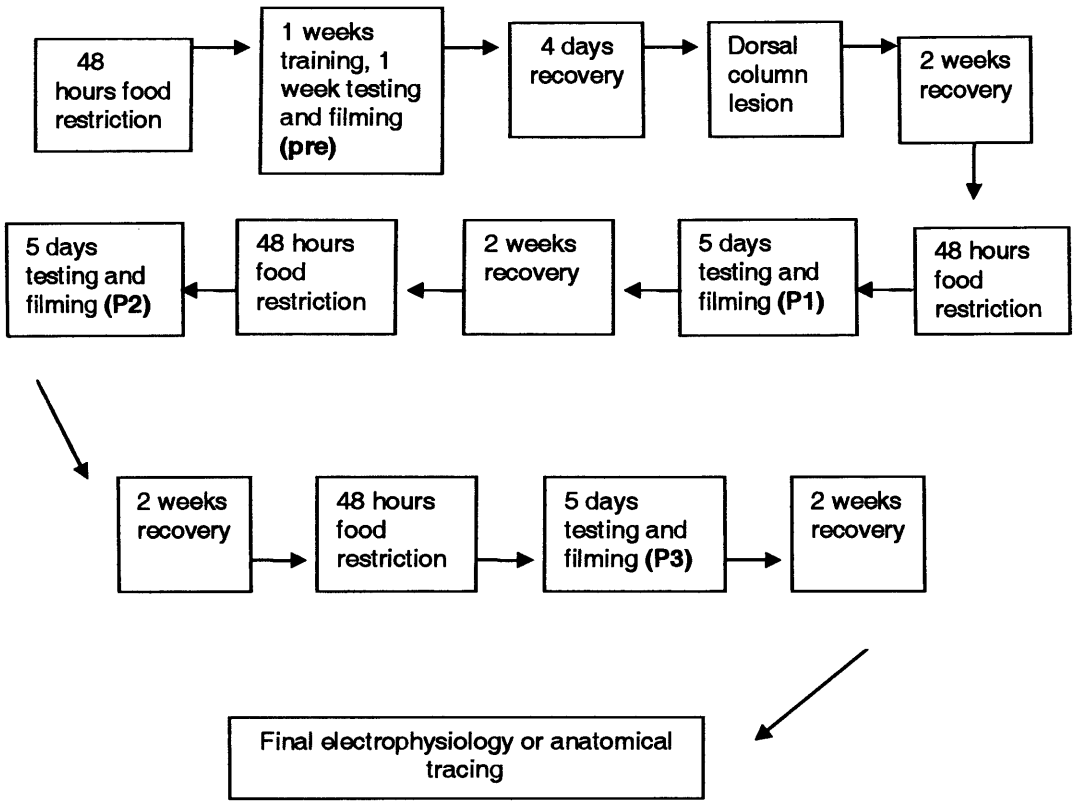


Figure 3.1 Time scale of the study.
Animals were trained to reach for the pellet before the lesion and underwent one testing session (5 days) pre-lesion and three testing sessions, each five days post-lesion. Filming of the three behavioural tests was carried out on the fifth day of each testing session. **Pre**: pre lesion testing session, **P1**: first post-lesion testing session, **P2**: second post- lesion testing session, **P3**: third post-lesion testing session.

3.2.2 Pellet retrieval test

Apparatus

The training box was a 40x40x12.5 box made of clear Perspex. In the centre of the front wall was a slit (width 1cm) that extended from 2cm above the floor to a height of 20cm (Figure 3.2). This slit was wide enough to allow the animal to insert its forelimb while carrying out a retrieval attempt. A movable shelf was attached to the outside of the front wall 3cm above the bottom of the box. The shelf was attached to the box with two removable screws on either side which allowed the experimenter to adjust

the gap between the placed pellet and the front of the box. This gap was such that the animal would have to reach with its forelimb, grasp the pellet, and lift it from the shelf, and also to prevent the animal from retrieving the pellet with its tongue. Two small indentations were made in the front of the shelf, opposite the slit, to hold the pellets. The positions of the indentations were such that the animal had to reach diagonally for the pellet, so that if the pellet was placed in the right indentation, the animal would retrieve it with its left paw and vice versa. A small mirror was place below the shelf at 45º to allow observation of the paw from below when analysing this test (see figure 3.3). Animals were filmed from the font, and during filming, a vertical mirror was placed on the same side as the preferred paw to provide a lateral view simultaneous with the frontal view of the grasping behaviour during this test (see figure 3.3).

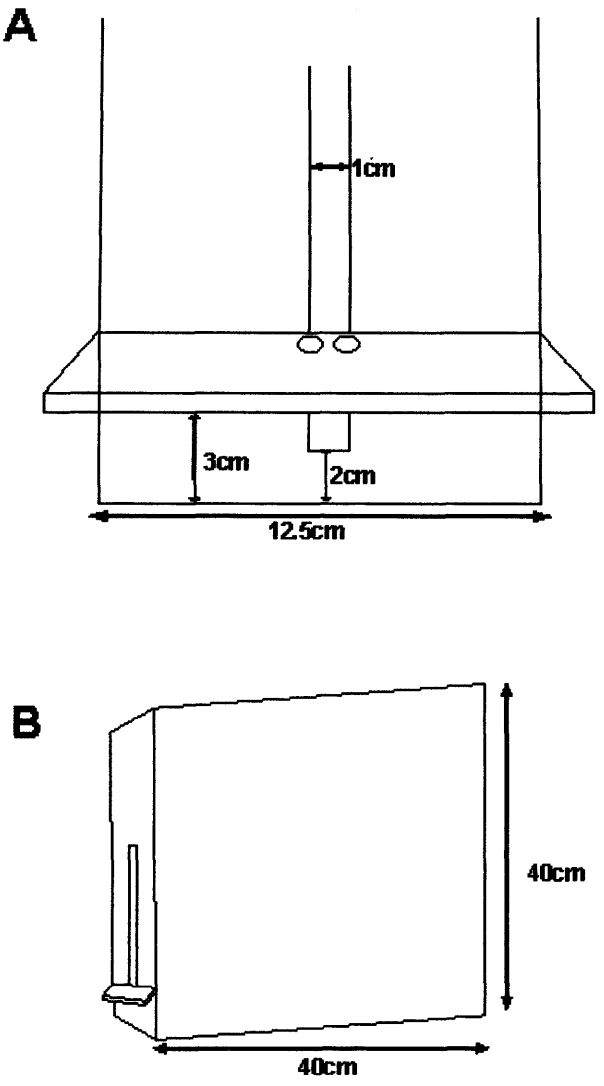


Figure 3.2 Schematic of training box for the pellet retrieval.
A clear Plexiglas box was used, shelf attached with two indentations for pellet placement. A mirror placed under the shelf at 45° allowed clear visualisation of the paw during grasping. **A** front view, **B** side view

Figure 3.3 Pellet retrieval test.
Selected video frame for an animal performing the pellet retrieval test. Mirrors placed below the shelf at 45° and on the same side as the preferred paw allow maximal visualisation of paw and body movements.

Food pellets

Initially pellets of standard rat and mouse diet (rat and mouse diet, B&K universal LTD, UK) were used weighing between 40 and 100mg. However, in the later stages of this study, precision pellets (chocolate and banana flavoured, 3.556mm diameter Lillico, UK) were used as we were unable to obtain the standard diet pellets.

Training and testing

Animals were trained for pellet retrieval test prior to the lesion, during the training week of the pre-lesion session. Animals were then tested for the pellet retrieval test during each day of the pre- and post-lesion testing sessions for 20 minutes. During the beginning of the pre-lesion training session, the shelf was placed close to the front of the box (<1cm) and a pellet was placed into each indentation so that the rat could grasp a pellet with either paw. The animal would reach for the pellet placed in the right indentation with its left paw, and to the left one with its right paw. The forepaw that was used most frequently was noted and defined as the animal's "preferred" paw. The animal was then "forced" to use its preferred paw by placing the pellet in the indentation contralateral to the preferred paw. During the training

session, the distance between the pellet and the front of the box was gradually increased, by moving the shelf, until it reached 2cm and was maintained at this distance for the remainder of the training week. Animals were tested during the pre- and post-lesion sessions with the shelf at 2cm and the success rate of pellet retrievals using the preferred paw was calculated. A successful reaching attempt was operationally defined as a reaching attempt that resulted in the animal reaching and contacting the pellet, successfully grasping it and bringing it back through the slit and consuming it. If the rat failed to grasp the pellet or if it dropped it then it was scored as an unsuccessful attempt. If the rat extended its forelimb through the opening but did not contact the pellet the reach was not counted. Success rates were calculated for reaches carried out when the shelf was 2cm away and were calculated as follows: *(Number of successful attempts/total number of attempts) x100.* The scores from all testing days where then combined for each session to provide an averaged success rate for each session and were displayed as a mean ± standard error of the mean.

3.2.3 Cylinder test

The cylinder test assesses forelimb use during spontaneous vertical exploration. When the animal rears onto its hindlimbs, it uses its forelimbs to support its weight and to maintain its balance (Figure 3.4A).

Apparatus

The cylinder was adapted from that used by (Liu *et al.*, 1999) and was made of clear Perspex, 5mm thick and measured 36cm in height and 20cm in diameter (Figure 3.4B).

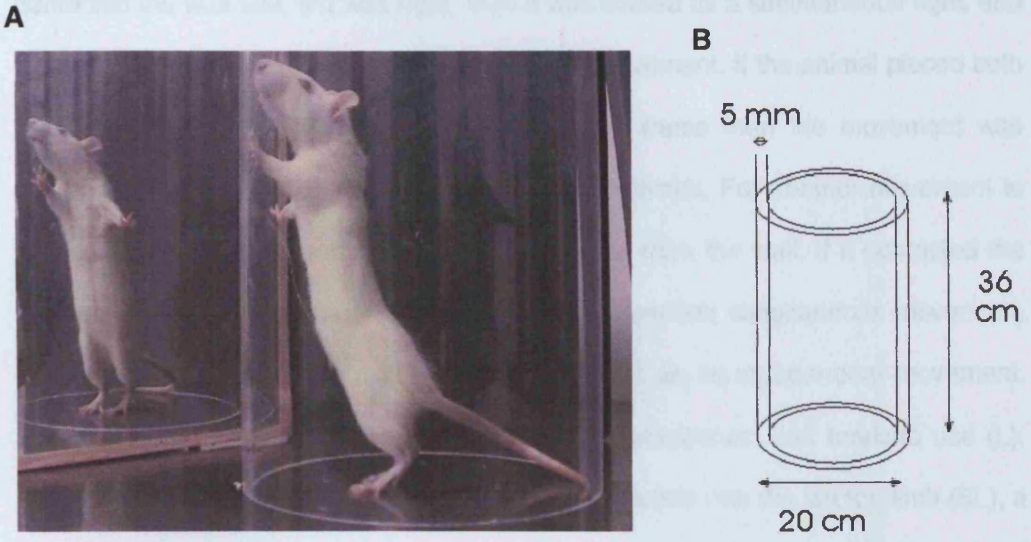


Figure 3.4 Cylinder test.
A Selected video frame of an animal performing the cylinder test. An angled mirror is placed to the side of the cylinder to allow maximal visualisation of forepaw contacts. **B** Schematic representation of the cylinder.

Filming and frame by frame analysis

Each animal was placed in the cylinder for 5 minutes, and filming was started as soon as the animal was placed in the cylinder so that no exploratory movements were missed. Animals were filmed from the front and during filming, an inclined mirror was placed at the side of the cylinder to allow the observation of all paw/cylinder contacts (see figure 3.4A). Frame by frame analysis was carried out at the end of the study. Forelimb use was observed during rearing and lateral exploration of the cylinder wall. The number of rears and the number of independent and simultaneous forelimb contacts were counted. As the animal started rearing, the first forelimb to contact the cylinder wall with weight support was scored as an independent movement of either left or right. A simultaneous movement was scored if the first limb maintained its position as the other forelimb contacted the cylinder wall. The second limb must have contacted the wall within one second of the first forelimb contact for it to have been scored as a simultaneous movement. It was noted which forelimb

contacted the wall first, if it was right, then it was scored as a simultaneous right, and if it was left it was scored as a simultaneous left movement. If the animal placed both forepaws onto the cylinder wall within one video frame then the movement was scored as a simultaneous movement with both forelimbs. For another movement to be scored, the animal must have removed its paw from the wall, if it contacted the cylinder wall within one second, it was scored as another simultaneous movement, and if it wasn't within a second then it was scored as an independent movement. Accordingly, forelimb usage was divided into five categories: Left forelimb use (L), right forelimb use (R), a simultaneous movement initiated with the left forelimb (SL), a simultaneous movement initiated with the right forelimb (SR), and a simultaneous movement with both forelimb placed on the cylinder wall within one video frame (SB). The percentage of each category of forelimb use was calculated to allow comparison between the pre- and post-lesion stages.

3.2.4 Sticker test

The sticker removal test is a sensorimotor test that requires some grasping ability and mobility.

Testing and filming

An orange sticker (13mm diameter) was placed on the bridge of the rat's nose (figure 3.5). The animal was placed in the same cylinder used for the cylinder test. This test was filmed along with the pellet retrieval test and cylinder test on the 5th day of each session. During filming, a mirror is placed on the side of the cylinder to allow visualisation of the sticker removal from all angles. For each test, the animal was given a maximum of 2 minutes to remove the sticker, and each animal was given 3 trials. Scoring measures were adopted from Diener and Bregman (1998). A score of

6 was given to the animal if it removed the sticker in one swift grasp, and zero was given if the animal did not attempt to remove the sticker (table 3.1)

Figure 3.5 Sticker removal test.
Selected video frame of an animal during the sticker removal test. A mirror is placed to the side of the cylinder to allow maximal visualisation of the animal when removing the sticker.

Score	Behaviour
0	No sticker removal attempted
1	Forelimb did not reach as high as the sticker
2	Forelimb reached as high as the sticker; but moved in a lateral plane, was unable to adduct actively and functionally towards the midline
3	Forelimbs reached as high as the sticker; head was contacted, but no sticker contact occurred
4	Sticker was contacted by the forelimb during the reaching attempt
5	Sticker was removed after multiple attempts but without a strong grasp
6	Sticker was tightly grasped and swiftly removed or pulled from the head

Table 3.1 Sticker removal test scoring table.
Scores from zero to six were used to a rate animal's performance during the sticker removal test.

3.2.5 Statistics

The quantitative results of the behavioural tests were analysed using SPSS (Statistical Program for Social Sciences, Chicago, IL) computer program. All graphs were produced using Origin 6.0 (Micrococal software Inc., MA, USA).

Pellet retrieval test

In the individual animal results, the data is displayed as a mean ± standard error of the mean for each session (see analysis section). Data for the group results are displayed as whisker and box plots displaying the mean and the maximum and minimum success rates. Differences were compared between the pre- and post-lesion sessions in all animals using a univariate analysis of variant. If variability existed within the stages ($P<0.05$), significant differences were determined using the Bonferroni correction which were reported at $P<0.05$ and $P<0.01$. For statistical purposes, the minimum total number of reaches accepted was *10 reaches per 20 minutes*, and animals must have performed for at least three days out of the five. If these criteria were not met, statistical significance was not calculated.

Cylinder test

Data for the cylinder test was represented as the percentage of forelimb usage for each of the categories previously described (see analysis section). The non parametric Friedman test (two way analysis on ranks) was used to determine significant differences within limb usage pre- and post-lesion. A minimum of *20 touches per 5 minutes* was used as the criteria for statistical analysis.

3.2.6 Histological evaluation of lesion site

Following perfusion and histological processing (see Chapter two section 2.25 for more details on histological processing) the cross sectional extent of the lesions was reconstructed using a Zeiss Axioskop microscope (Zeiss, W. Germany) and a drawing tube. A reference section was chosen and the outline was drawn using dark field. Using both dark field and light field, tissue destruction, gliosis, and scarring was outlined in a series of sections that best represented the lesion. The final outline of the lesion was summed up from the reconstructed sections.

3.3 Results

3.3.1 Behavioural observations

Histological reconstructions demonstrated that the lesion extent with respect to the dorsal columns (DCs) varied between the animals. In order to carry out comparisons between lesion size and behavioural outcome, animals were divided into three groups according to the size of their lesion: 1. incomplete dorsal column lesion, resulting in the sparing of some of the CST, 2. complete dorsal column lesion with no damage to the DLF, where the rubrospinal tract (RST) is located, as was demonstrated in BDA tracing of the RST (See chapter 4), 3. complete dorsal column lesion but with extension to the DLF and/or the ventral white matter, resulting in damage to the RST and the ventral corticospinal tract (vCST)

Twenty animals were used in this study: seven had an incomplete DC lesion sparing some of the main component of the CST which is located in the ventral part of the dorsal columns, four animals had a lesion specific to the dorsal columns (DC), with

no damage to the RST or the vCST, and nine had a lesion that destroyed the DCs and also extended to either the DLF or the vCST or both (Figure 3.6).

The results of this chapter are divided into two main sections, the first shows the results from the pellet retrieval test, cylinder test and sticker removal test for individual animals. The results appear in order of animal number to assist the reader in cross reference. The second shows group results of the reaching test according to the size of the lesion.

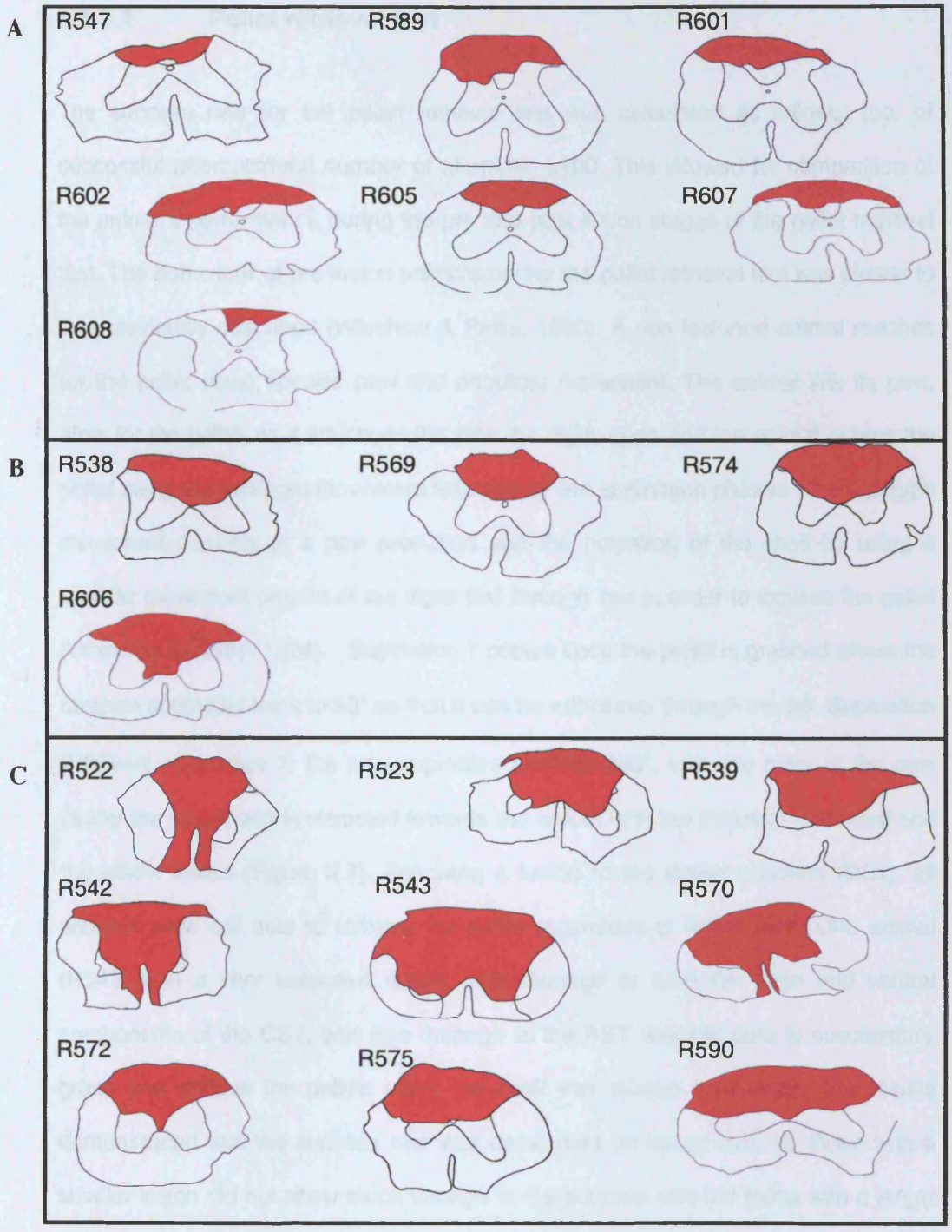


Figure 3.6 Histological reconstructions of spinal cord lesions.
A Incomplete dorsal column lesion, **B** Specific lesion to the dorsal columns, **C** Dorsal column lesion with extension to the DLF and/or ventral CST.

3.3.1.1 Pellet retrieval test

The success rate for the pellet retrieval test was calculated as follows: (no. of successful attempts/total number of attempts) x100. This allowed for comparison of the animal's performance during the pre and post lesion stages of the pellet retrieval test. The behaviour of pre lesion animals during the pellet retrieval test was similar to that previously described (Whishaw & Pellis, 1990). A non lesioned animal reaches for the pellet using specific paw and shoulder movement. The animal lifts its paw, aims for the pellet, as it advances the paw the digits open and the animal grasps the pellet using the arpeggio movement followed by two supination phases. The arpeggio movement consists of a paw pronation and the palpation of the shelf by using a specific movement pattern of the digits five through two in order to localise the pellet (Whishaw & Gorny, 1994). Supination 1 occurs once the pellet is grasped where the forepaw supinates back to 90° so that it can be withdrawn through the slit. Supination 2 follows supination 1; the paw supinates towards 180°, with the palm of the paw facing the mouth and is retracted towards the mouth with the shoulder extended and the elbow flexed (Figure 3.7). Following a lesion to the dorsal columns (DCs), all animals were still able to retrieve the pellet regardless of lesion size. One animal (R543) with a very extensive lesion, with damage to both the main and ventral components of the CST, and also damage to the RST was still able to successfully grasp and retrieve the pellets when the shelf was placed 1cm away. The results demonstrated that the success rate was dependant on lesion size, as those with a smaller lesion did not show much change in the success rate but those with a larger one did. Although following the lesion, the animals were able to carry out successful retrievals; the strategy of retrieving the pellet was different, with the arpeggio and supination 1 and 2 phases absent (Figure 3.8). Once the pellet is grasped, the animal drops its limb and the head is lowered to consume the food. Abnormal rotary movements were seen when the animal reached for the pellet, with the ipsilateral

shoulder dropping in order for the animal to maintain its balance (Figure 3.8A). It was also noted, during the pellet retrieval test that the animals sometimes did not seem to be aware of the pellet in their forepaw which led them to dropping the pellet and not scoring a successful trial (Figure 3.9). In addition to this, the animals often reached for the pellet, missed, and carried on to bring their paw to their mouth, apparently not realising that there was no food pellet in their paw (Figure 3.10). This occurred in all animals with a lesion to the dorsal column except for R608.

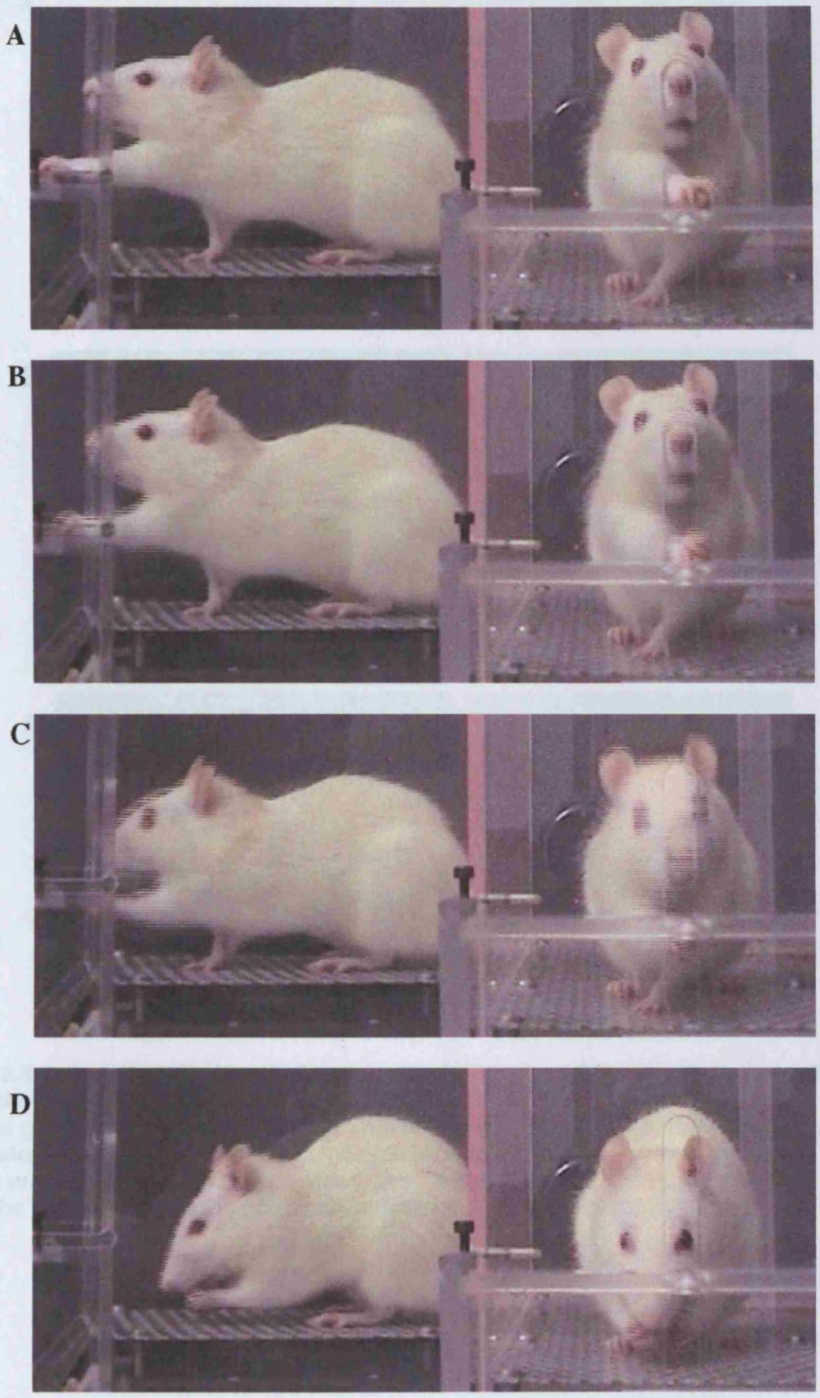


Figure 3.7 Selected video frames for an animal (R575) performing the pellet retrieval task pre lesion.
In this figure and subsequent ones, each frame shows a frontal view of the animal and a side view as reflected in a mirror placed on the same side as the preferred paw used during the retrieval. **A** Animal grasping the pellet, **B** Supination 1, **C** Supination 2, **D** Pellet release and consumption.

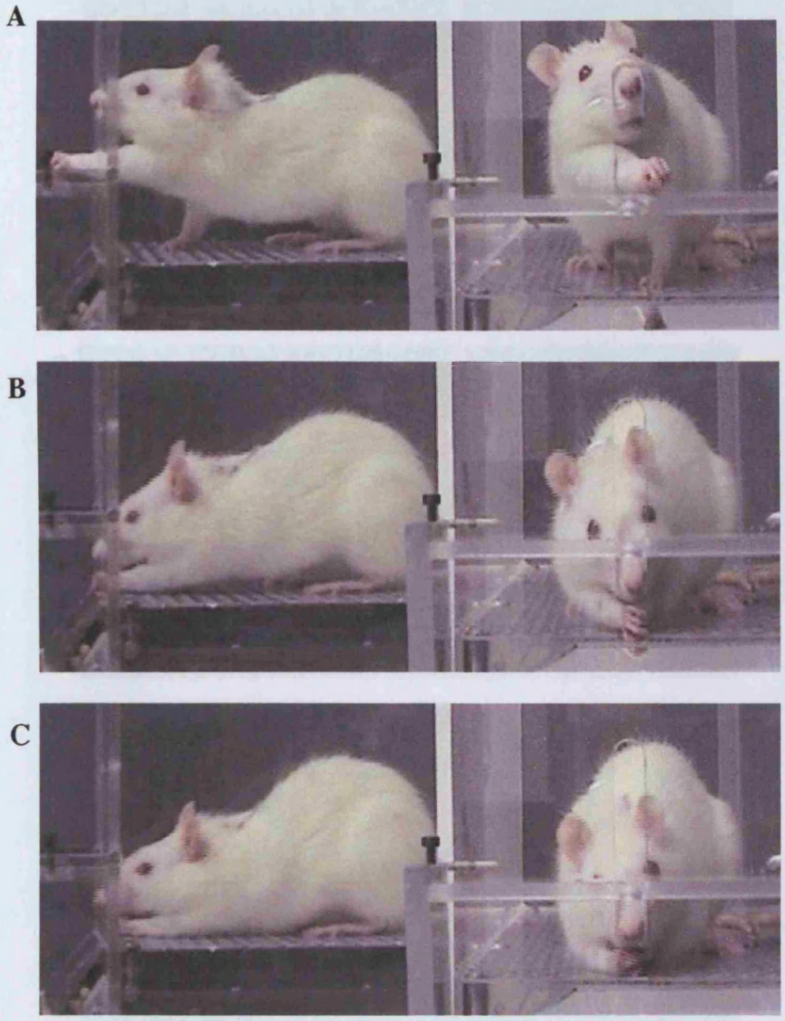


Figure 3.8 Selected video frames for an animal (R575) performing the pellet retrieval test during the first post lesion stage
(A) Pellet grasped and lifted from the shelf, the animal's body and head is twisted during grasping,
(B) Ipsilateral shoulder has dropped as the animal bring the forepaw towards the mouth,
(C) The animal's has dropped (side view), the head is lowered in order to consume the pellet. During the post lesion grasping behaviour, supination 1 & 2 phases are missing.

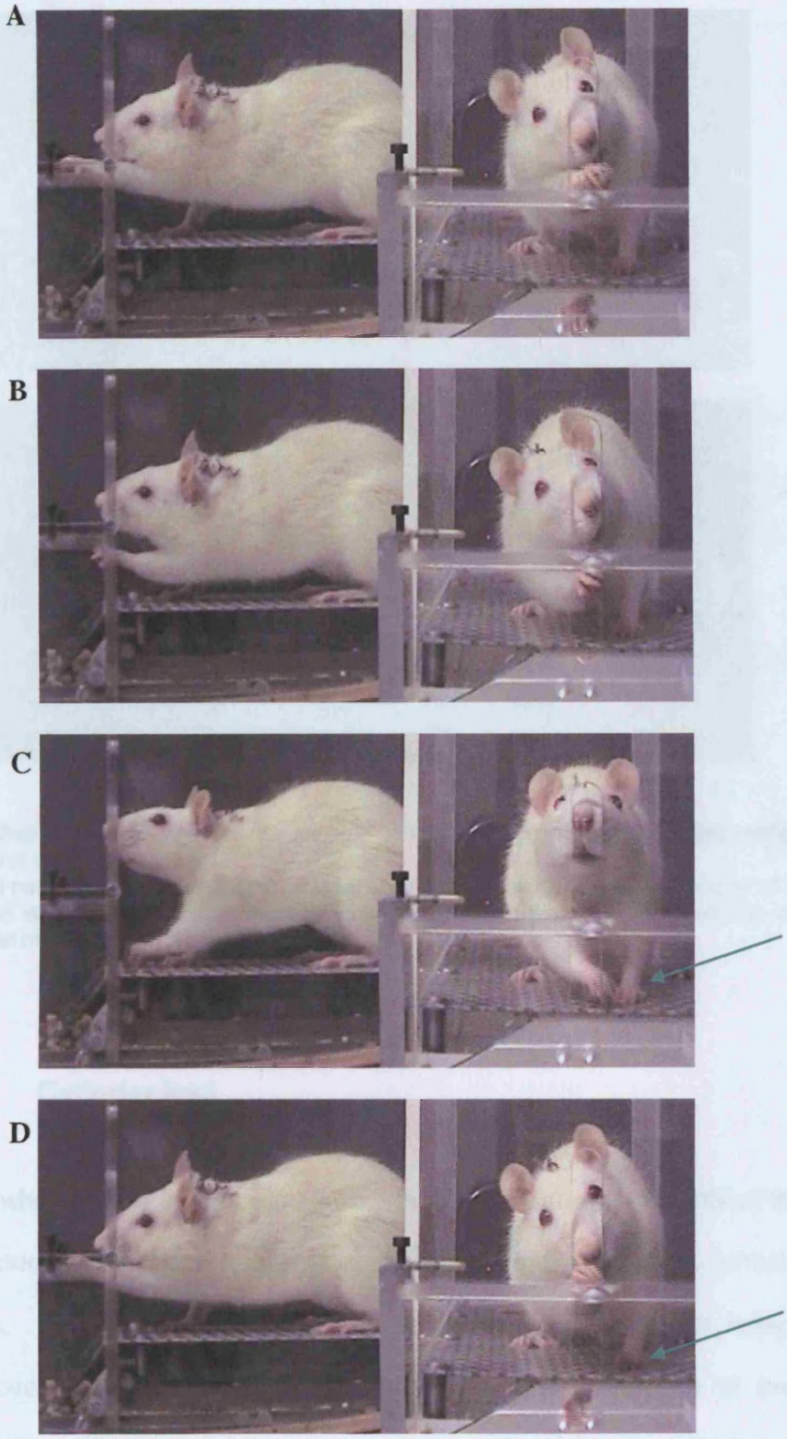


Figure 3.9 Selected video frames for an animal (R575) performing the pellet retrieval test during the first post lesion stage.
A & B Animal grasping and retrieving the pellet, **C** The pellet has been dropped (arrow) perhaps because the animal did not realise it succeeded in picking it up, **D** Animal reaching for the next pellet that has not yet been placed by the experimenter. Arrow clearly shows the pellet that had been dropped.

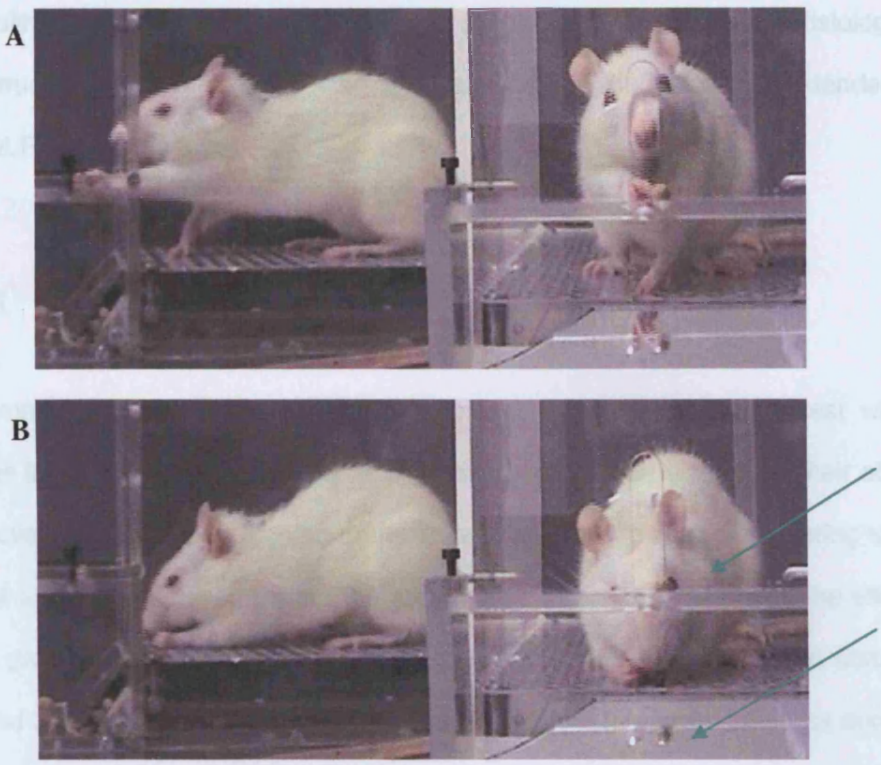


Figure 3.10 Selected video frames for an animal (R575) performing the pellet retrieval test during the first post lesion stage.
A The animal reaches for the pellet and misses, **B** Animal takes its paw to its mouth not realising that the pellet was missed. Arrows clearly shows the missed pellet as seen on the shelf and reflected in the mirror.

3.3.1.2 Cylinder test

All animals when placed in the cylinder spontaneously reared and explored the walls of the cylinder using both forepaws, either independently or as simultaneous movements. The percentage of forepaw usage was calculated for independent forepaw movements and simultaneous ones. The paw preference of the pellet retrieval test in pre lesion animals was not always reflected in the cylinder as some animals made contacts using the non-preferred forepaw more frequently than with the preferred one, although non significantly. Following a bilateral dorsal column lesion, the percentage of forelimb usage did not change significantly in any of the forelimb categories. The exception to this was R602, where the left paw was not

used either in an independent or in a simultaneous movement. Histological reconstruction of the lesion site later demonstrated that the lesion had extended to the L-DLF (see figure 3.26).

3.3.1.3 Sticker test

All animals were tested for the sticker removal test, a sensorimotor test which requires full range of motion and grasping ability. Animals were rated on their ability to remove the sticker by assigning them scores from zero to six, zero referring to no attempt at sticker removal. During the sticker test, the animals removed the sticker during grooming motions. Pre lesion animals reached for the sticker and abruptly removed it from the head using a single forepaw or both forepaws. This was done in either a single (score 6) or multiple attempts (score 5). Following injury and independent of lesion size, animals were still able to remove the sticker either in a single attempt or after multiple attempts obtaining either a score of 5 or 6.

3.3.2 Results for individual animals

R522- Right paw preference

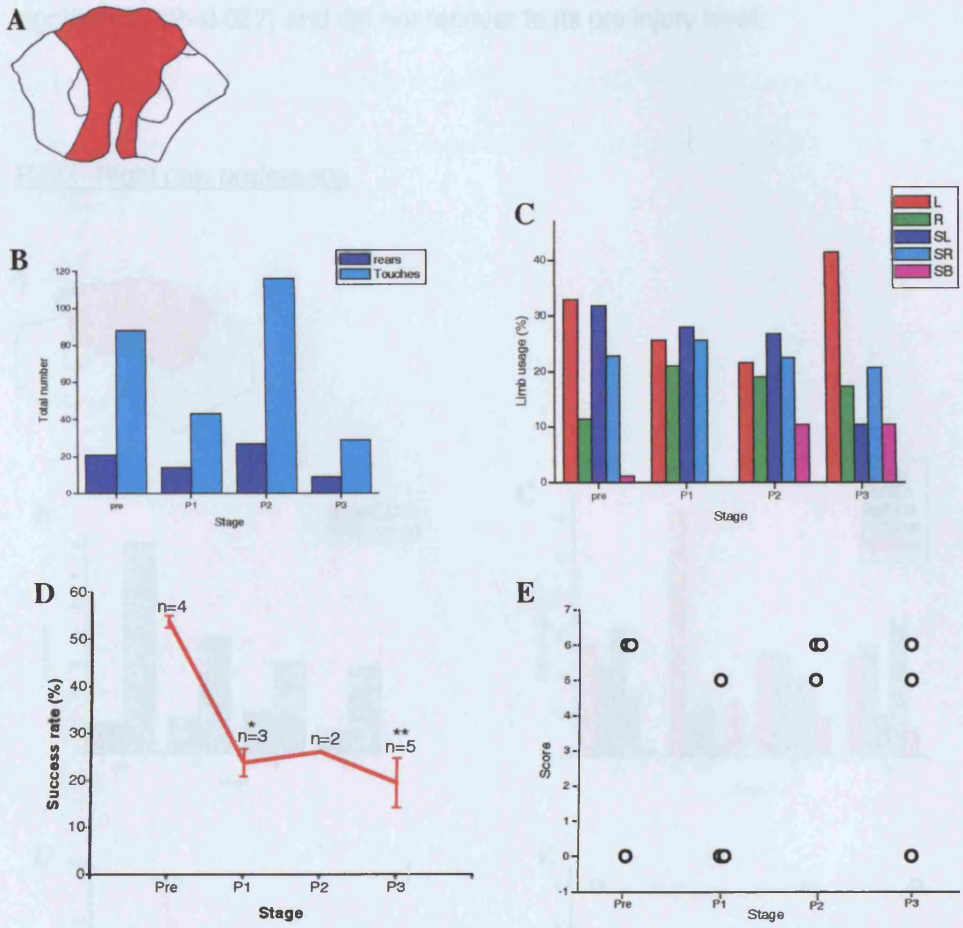


Figure 3.11 Behavioural data for R522.
A Reconstruction of lesion, right side of the cord is on the right, and left is on the left; **B &C** data from cylinder test illustrating the percentage of forelimb usage, L=independent use of left, R= independent use of right, SL= simultaneous movement initiated with left paw, SR= simultaneous movement initiated with right paw, SB= simultaneous movement with both paws placed on cylinder wall within one video frame; **D** data from pellet retrieval test; **E** data from sticker test. Error bars represent standard error of the mean, n=number of days where the animal performed well, meeting the criteria required for statistical analysis. Standard error calculated only if $n \geq 3$. (*= $p \geq 0.05$, **$p \geq 0.001$ as compared to control)

The lesion spanned from caudal C5 to rostral C6. Histological reconstruction demonstrated that it extended ventrally and destroyed the v-CST as well as the DCs. Although this animal showed a right paw preference for the reaching test, it showed a

different paw preference during all stages of the cylinder test. There was a drop in the number of touches during the cylinder test at P1, but a recovery to a higher level at P2, then another drop at P3. In the pellet retrieval test, the success rate dropped significantly (P=0.027) and did not recover to its pre injury level.

R523- Right paw preference

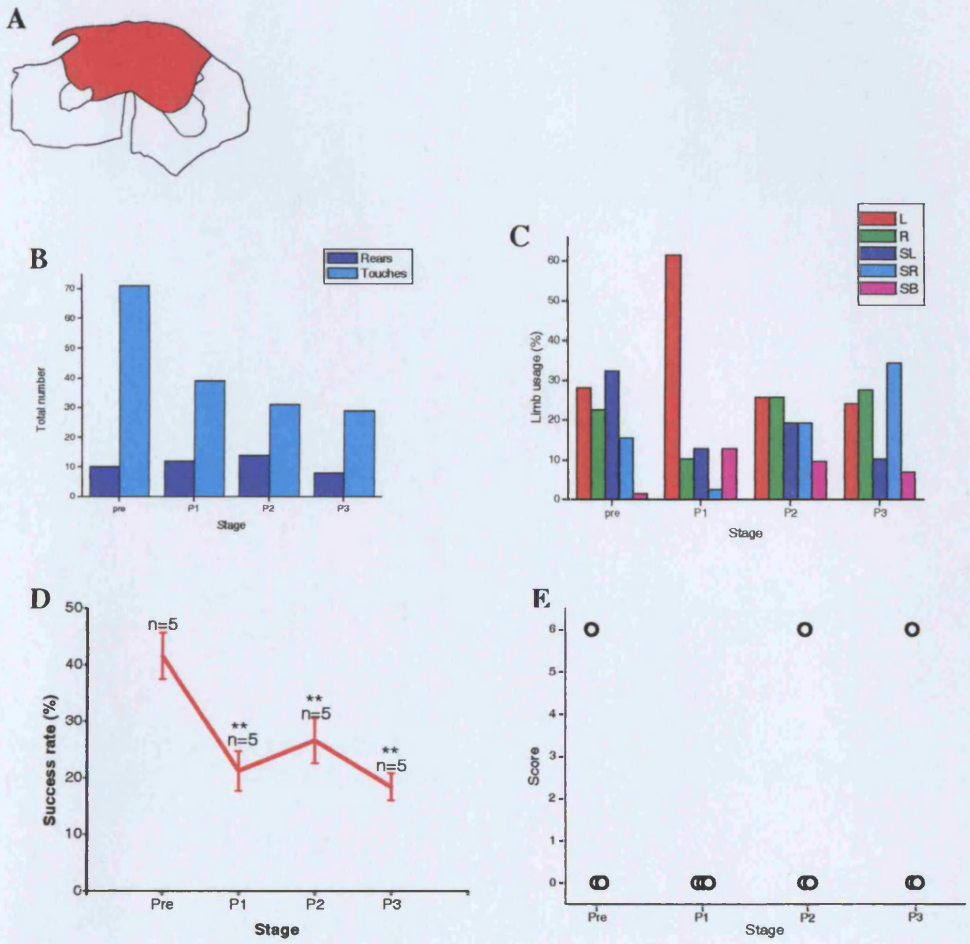


Figure 3.12 Behavioural data for R523.
A Reconstruction of lesion; **B & C** data from cylinder test; **D** data from pellet retrieval test; **E** data from sticker test. Format as figure 3.11.

The lesion spanned from rostral C5 to caudal C6. Histological reconstruction demonstrated that it extended slightly to both the left and right DLF. The cylinder test showed a consistent fall in the number of touches made by this animal. Pre-injury, the animals used both left and right paw at a similar rate. However, following the lesion, the animal used its non-preferred paw more but then reverted back to using both at P2 and P3. The pellet retrieval test showed a significant drop in success rate (P=0) without any recovery.

R538- Right paw preference

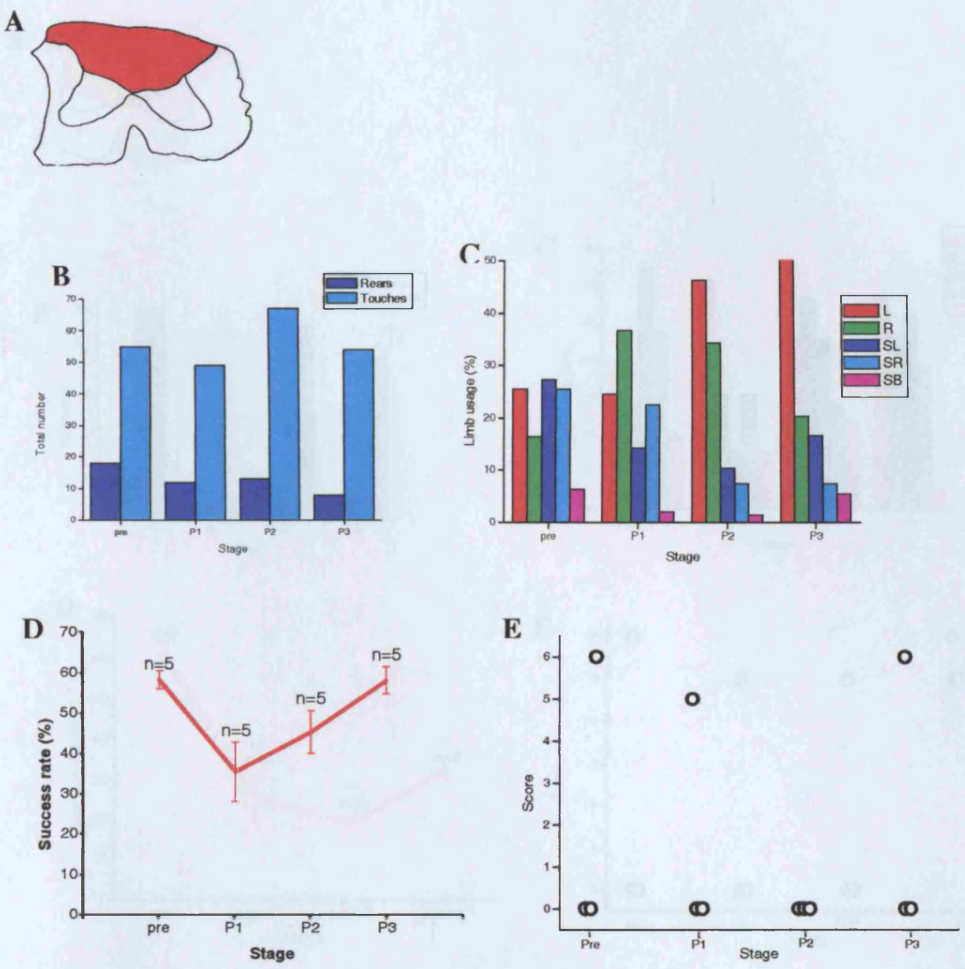


Figure 3.13 Behavioural data for R538.
A Reconstruction of lesion; **B & C** data from cylinder test; **D** data from pellet retrieval test; **E** data from sticker test. Format as figure 3.11.

The lesion spanned from caudal C3 to mid C5. Histological reconstruction demonstrated that it was specific to the DCs and did not destroy the v-CST or the DLF. In the cylinder test, this animal showed a trend to using the non-preferred paw over the preferred one. The total number of touches did not change much after the lesion. Although there was a drop in the success rate of the pellet retrieval test following the lesion (non-significant), the animal recovered back to its pre-injury level.

R539- Left paw preference

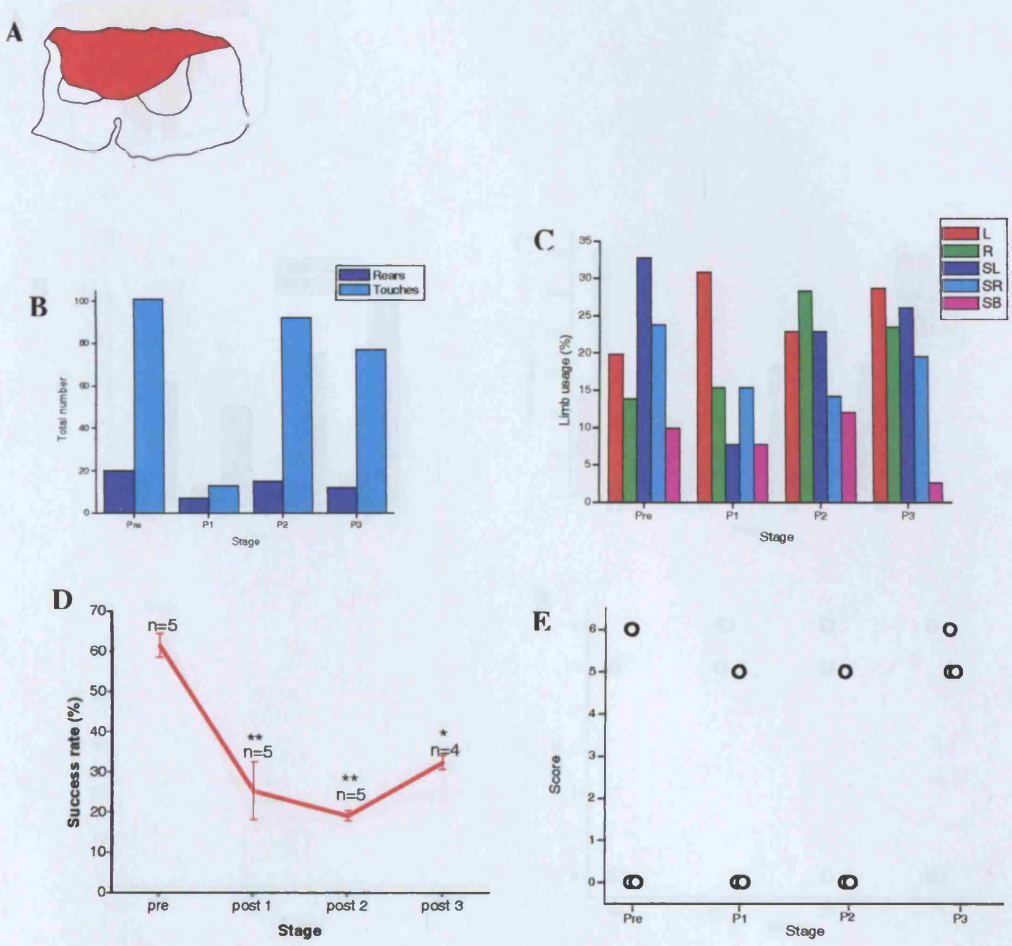


Figure 3.14 Behavioural data for R539.
A Reconstruction of lesion; **B & C** data from cylinder test; **D** data from pellet retrieval test; **E** data from sticker test. Format as figure 3.11.

The lesion spanned from rostral C4 to caudal C4. Histological reconstruction demonstrated that it extended slightly to both the left and right DLF. The cylinder test showed a large drop in the number of touches (less than 20) which increased to almost baseline at P2. The success rate of the pellet retrieval test dropped significantly (P=0) and remained low without any significant recovery.

R542- Left paw preference

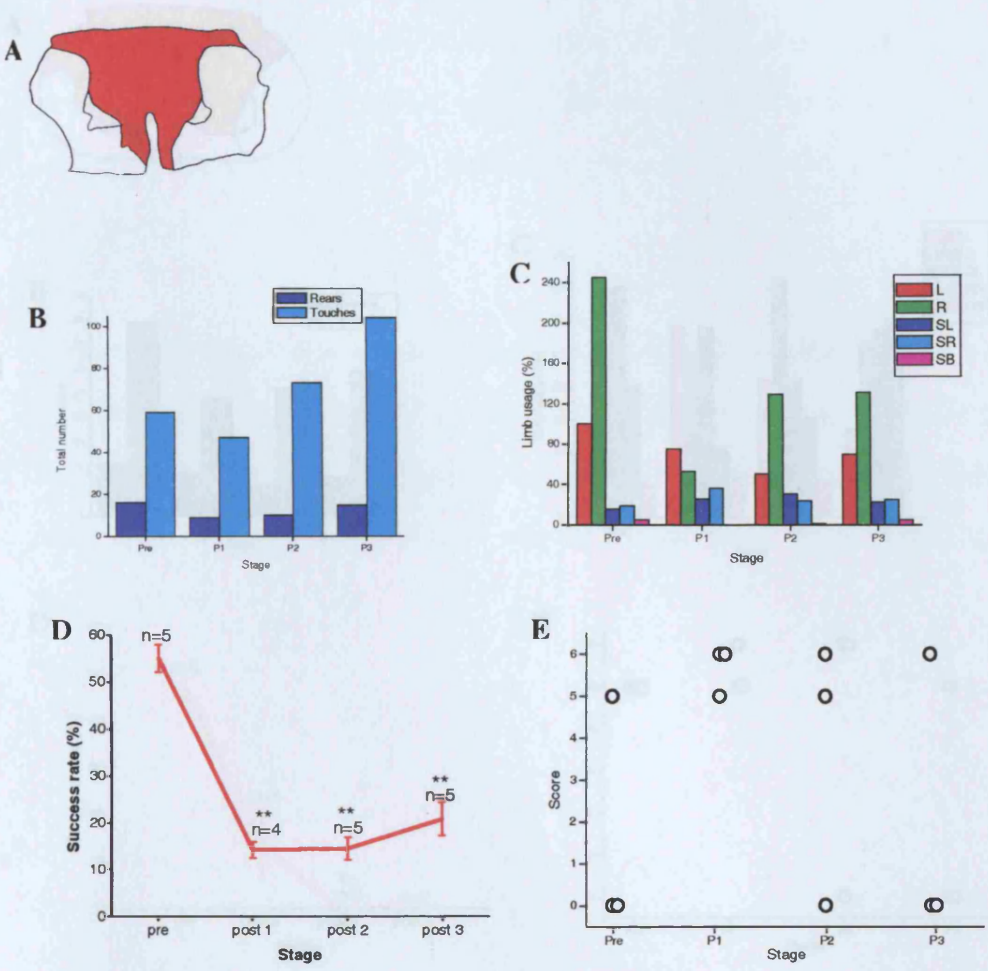


Figure 3.15 Behavioural data for R542.
A Reconstruction of lesion; **B & C** data from cylinder test; **D** data from pellet retrieval test; **E** data from sticker test. Format as figure 3.11.

The lesion to this animal spanned from mid C4 to mid C5 and histological reconstruction demonstrated that it extended to the v-CST. The number of touches made during the cylinder test slightly reduced during P1 but then increased at P2, and even more at P3. The non-preferred paw was used to contact the cylinder more frequently than the preferred paw. Following the lesion, the success rate of the pellet retrieval test dropped significantly (P=0) and did not recover.

R543- Right paw preference

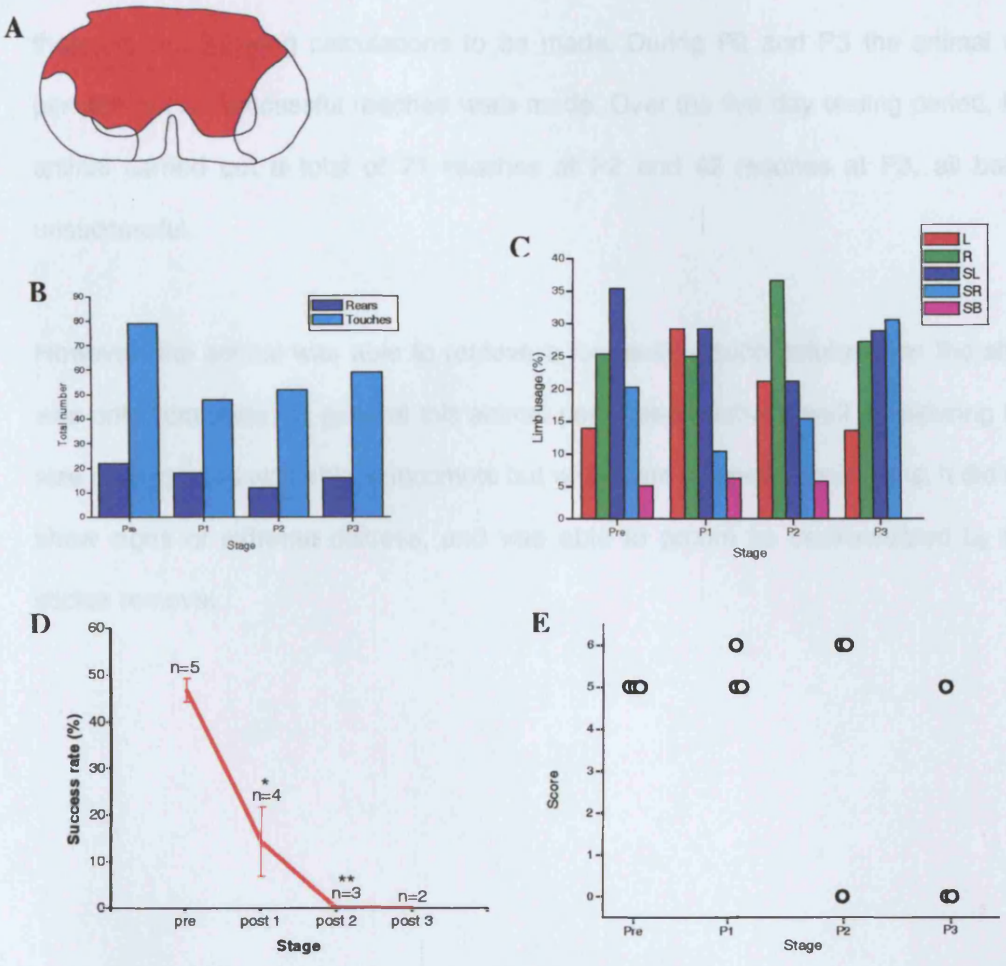


Figure 3.16 Behavioural data for R543.
A Reconstruction of lesion; **B & C** data from cylinder test; **D** data from pellet retrieval test; **E** data from sticker test. Format as figure 3.11.

The lesion spanned from rostral C4 to mid C5. This animal had a severe lesion spreading to the DLF on the both sides of the cord, and the v-CST, with only a small amount of DLF and ventrolateral matter spared. Although the lesion was very extensive, there was little affect on the total number of touches as shown in the cylinder test. However, during the pellet retrieval test, the success rate of the reaching test fell from 46.6% to 14.25% at P1 and fell even lower after that (zero).

During P3, the animal's performance did not meet the criteria required for statistical measurements (minimum of 10 reaches and a minimum of three days performance), therefore not allowing calculations to be made. During P2 and P3 the animal did perform but no successful reaches were made. Over the five day testing period, the animal carried out a total of 71 reaches at P2 and 42 reaches at P3, all being unsuccessful.

However, the animal was able to retrieve a few pellets successfully when the shelf was only 1cm away. In general this animal performed relatively well considering the size of lesion and was able to locomote but with some balance impairments. It did not show signs of extreme distress, and was able to groom as demonstrated by the sticker removal.

R547- Right paw preference

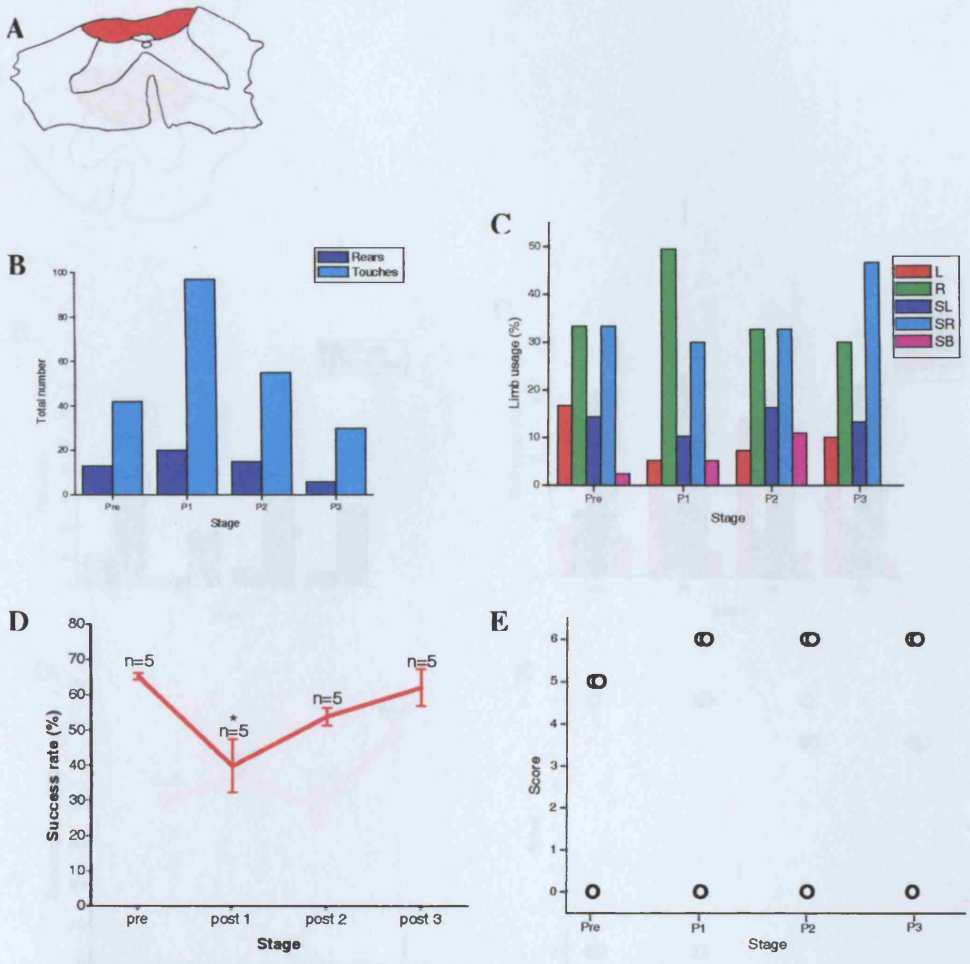


Figure 3.17 Behavioural data for R547.
A Reconstruction of lesion; **B & C** data from cylinder test; **D** data from pellet retrieval test; **E** data from sticker test. Format as figure 3.11.

The lesion spread from mid C4 to caudal C5. Histological reconstruction revealed that it was an incomplete lesion which spared a small amount of white matter in the DCs, therefore sparing some of the CST. The number of touches in the cylinder test actually increased following the lesion, and then dropped slightly again. Although the main CST was not completely severed, the success rate of the pellet retrieval test did drop significantly (P=0.007) following the lesion, but then recovered by P3.

R569- Right paw preference

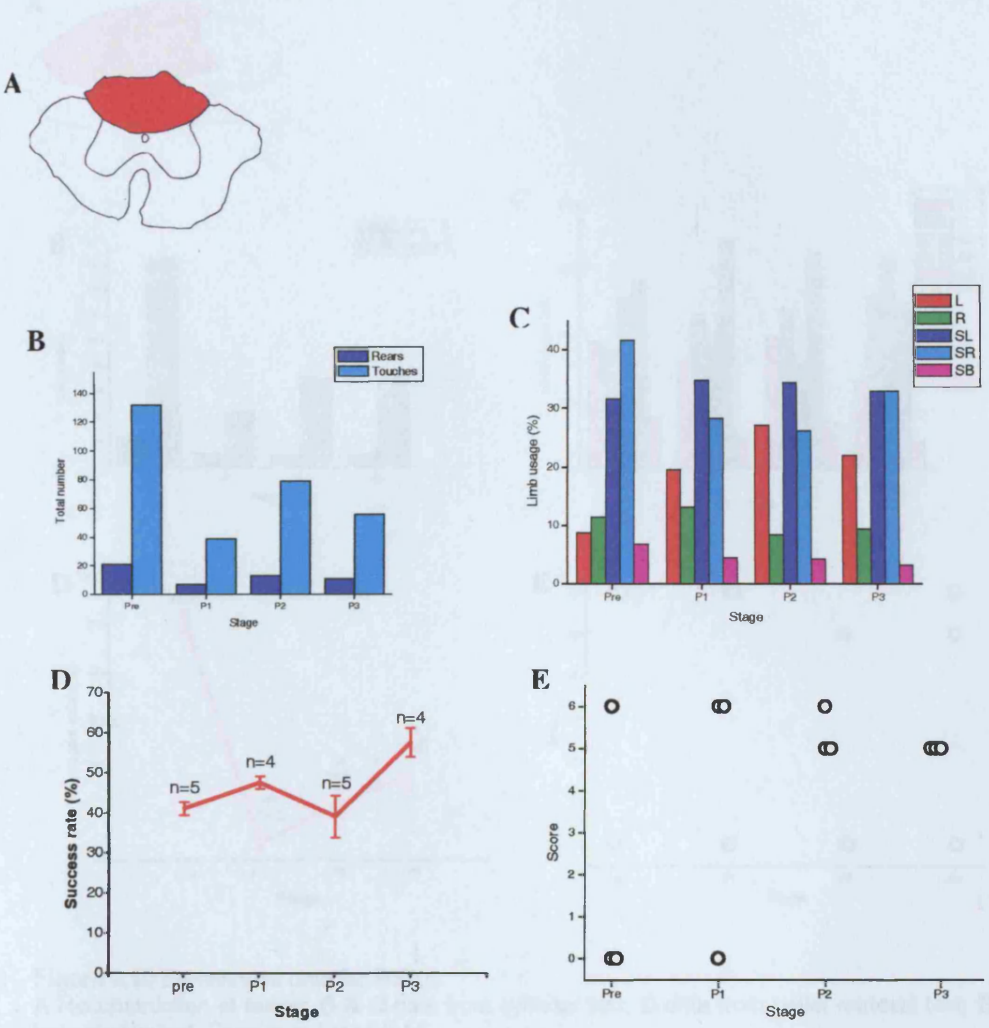


Figure 3.18 Behavioural data for R569.
A Reconstruction of lesion; **B & C** data from cylinder test; **D** data from pellet retrieval test; **E** data from sticker test. Format as figure 3.11.

The lesion was made at caudal C4 with minimal spread to the white matter outside the dorsal columns. During the cylinder test, the number of touches decreased following the lesion, but then increased at P2, and fell again at P3. While contacting the cylinder walls, the animal used its non-preferred paw after the lesion more than the preferred one. In the pellet retrieval test, the success rate actually increased slightly after the lesion, then decreased at P2, and increased again at P3.

R570- Left paw preference

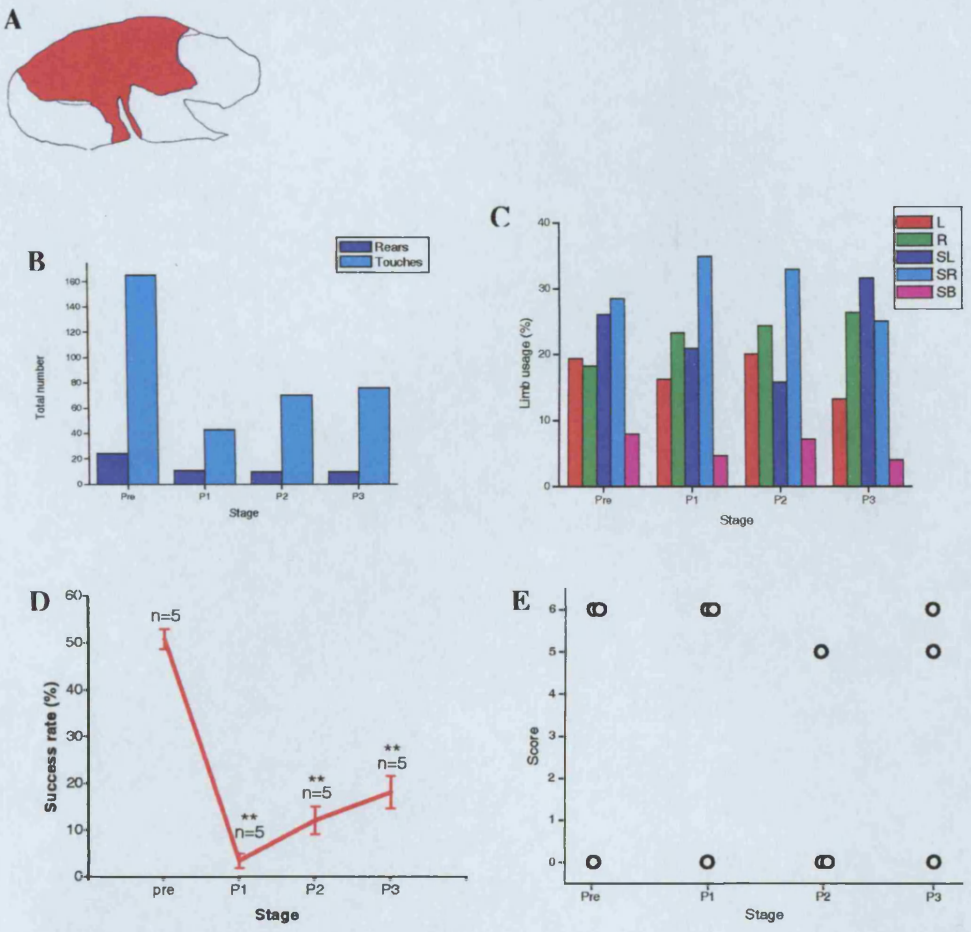


Figure 3.19 Behavioural data for R570.
A Reconstruction of lesion; **B & C** data from cylinder test; **D** data from pellet retrieval test; **E** data from sticker test. Format as figure 3.11.

The lesion was made at rostral C5 and histological reconstruction demonstrated that it extended beyond the DCs into the v-CST and the L-DLF. The number of contacts to the cylinder decreased from 165 to 43 and only recovered slightly at P2 and P3. The success rate of the pellet retrieval test decreased significantly (P=0) following the lesion and did not recover to its original level.

R572- Right paw preference

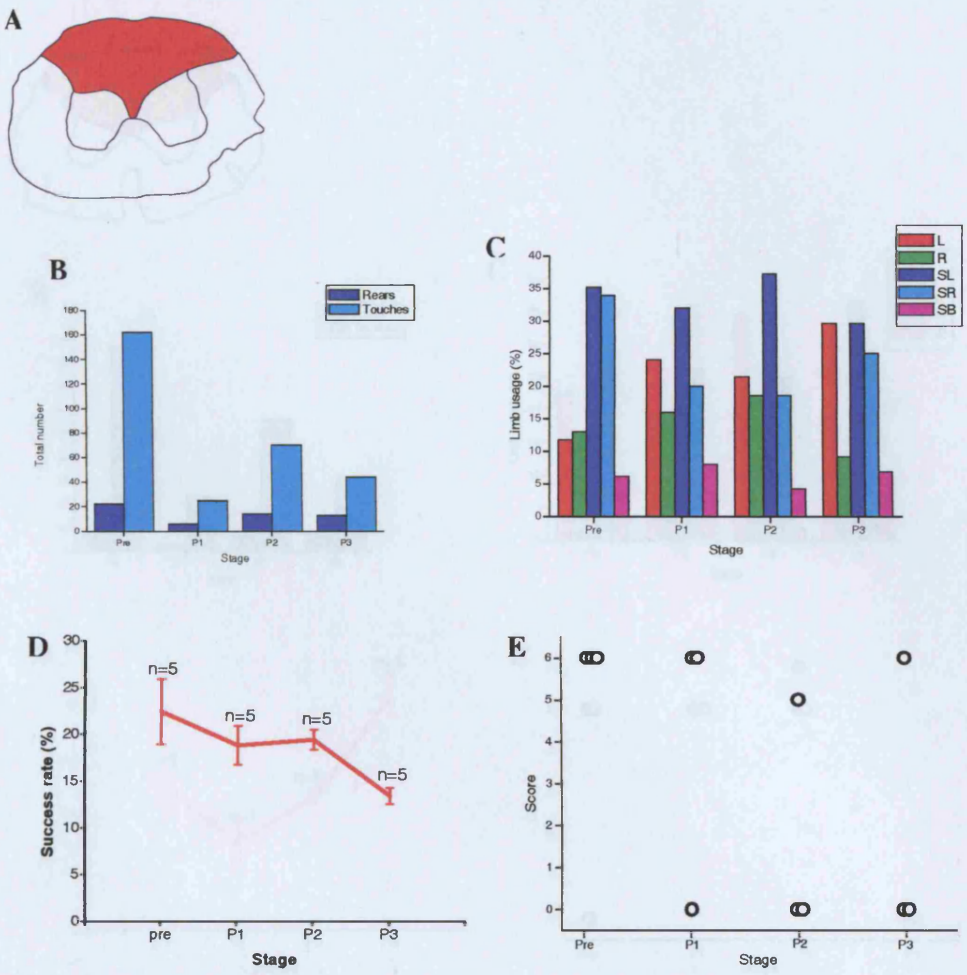


Fig. 3.1 Behavioural data for R572.
A Reconstruction of lesion; **B & C** data from cylinder test; **D** data from pellet retrieval test; **E** data from sticker test. Format as figure 3.11.

The lesion to this animal was at C5 and extended slightly to the DLF on both sides, as shown by the histological reconstruction. The number of touches in the cylinder test decreased from 162 to 25 and only recovered slightly at P2 and P3. The success rate of the pellet retrieval test decreased, although not significantly (From 22.4 to 13.4), and did not recover to its pre-lesion rate.

R574- Left paw preference

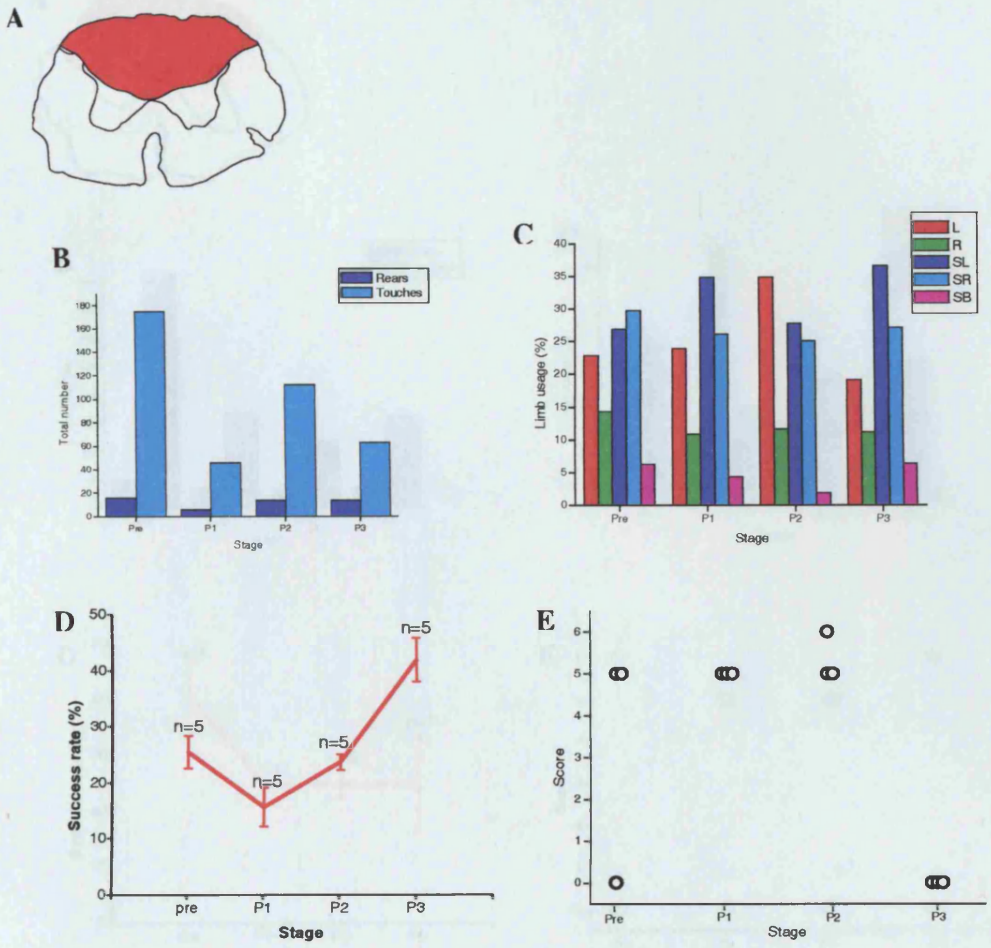


Figure 3.20 Behavioural data for R574.
A Reconstruction of lesion; **B & C** data from cylinder test; **D** data from pellet retrieval test; **E** data from sticker test. Format as figure 3.11.

The lesion spanned from caudal C4 to caudal C5 with no extension into the DLF or ventral white matter. During the cylinder test, the number of touches decreased from 175 to 46, but increased to112 at P2 with another slight decrease at P3. The success rate of the pellet retrieval test decreased after the lesion, but then recovered to a rate higher than the pre-lesion rate.

R575- Right paw preference

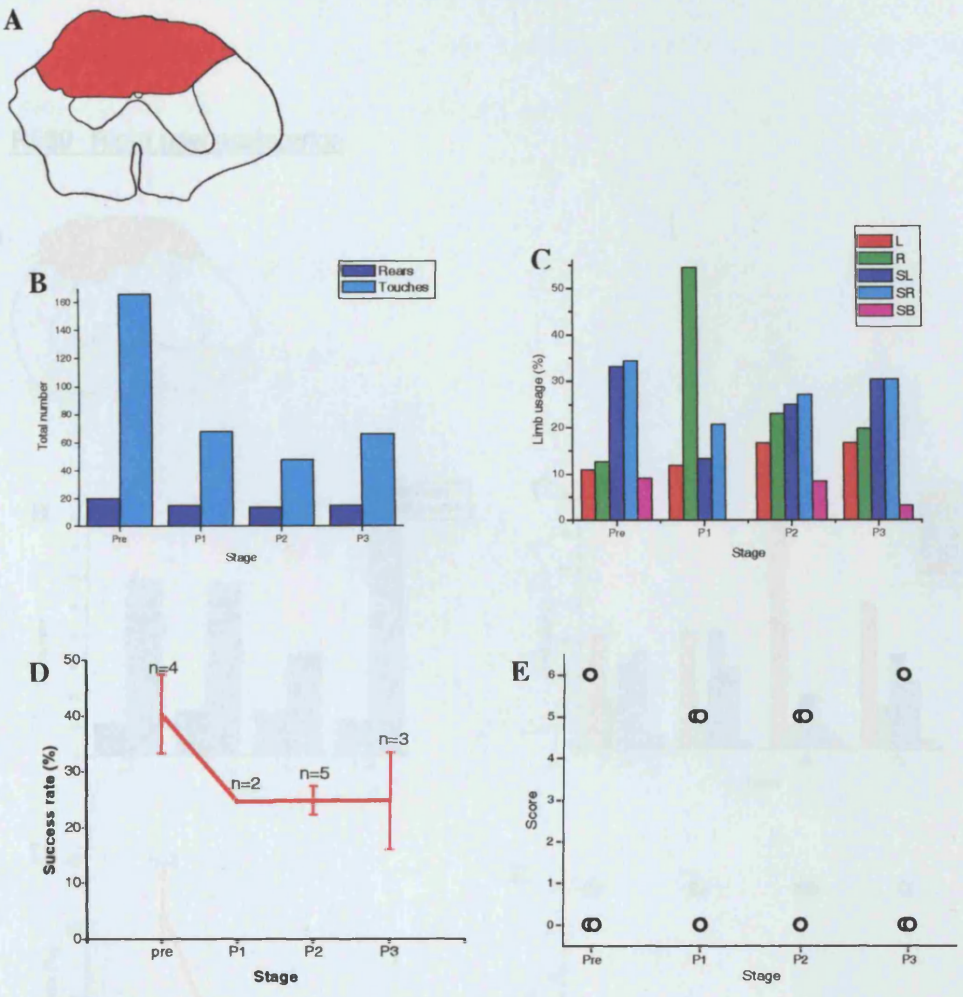


Figure 3.21 Behavioural data for R575.
A Reconstruction of lesion; **B & C** data from cylinder test; **D** data from pellet retrieval test; **E** data from sticker test. Format as figure 3.11.

In this animal, the lesion spanned from caudal C4 to rostral C5 and extended to the L-DLF, and slightly to the R-DLF. The number of touches decreased and remained at a low level. Following the lesion, the animals used its non-preferred paw (Left) more frequently than the preferred and reverted to using both paws equally at P2 and P3. Success rate of the pellet retrieval test also decreased after the lesion, although only slightly. During P1, the animal only performed on two days out of the five, as this

does not meet the criteria for statistical measurements, no statistical calculations were carried out.

R589- Right paw preference

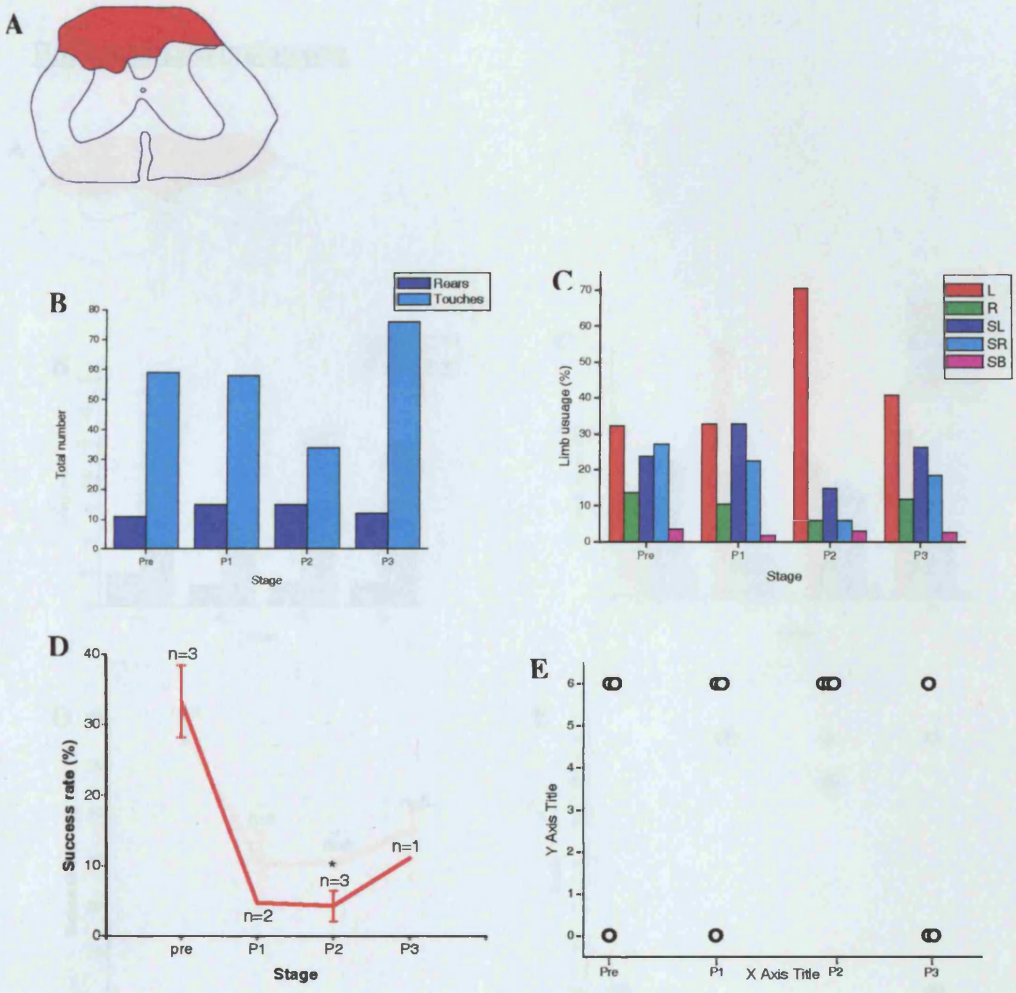


Figure 3.22 Behavioural data for R589.
A Reconstruction of lesion; **B & C** data from cylinder test; **D** data from pellet retrieval test; **E** data from sticker test. Format as figure 3.11.

The lesion was at rostral C5 and did not destroy all of the DCs, leaving most of the CST spared but part of the L-DLF was damaged. The number of touches remained fairly constant over the three stages and the animal used its non-preferred paw more

than its preferred paw during all stages of the assessment. During the pellet retrieval test, the success rate decreased at P1 and only recovered slightly by P3. This animal only preformed on two days out of the five at P1, and only performed once at P3, therefore not allowing statistical calculations to be carried out.

R590- Left paw preference

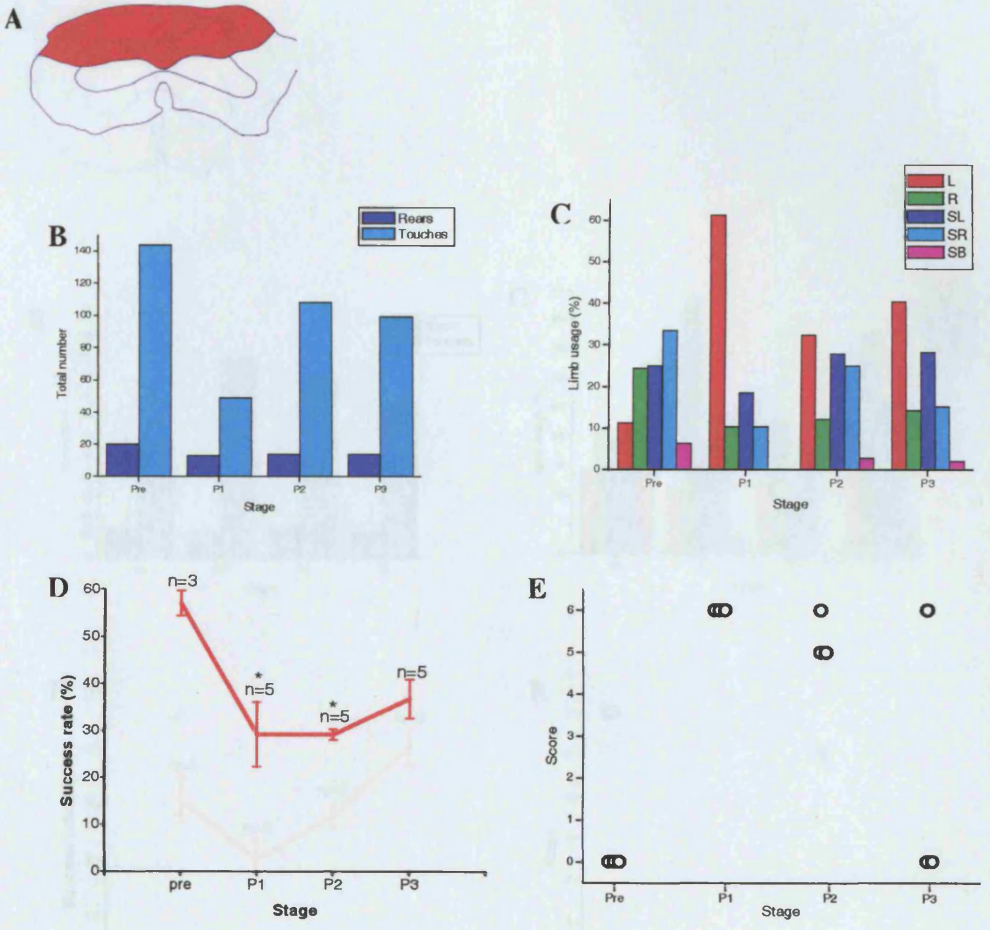


Figure 3.23 Behavioural data for R590.
A Reconstruction of lesion; **B & C** data from cylinder test; **D** data from pellet retrieval test; **E** data from sticker test. Format as figure 3.11.

The lesion was found to be at caudal C4 and extended beyond the DCs to both the right and left DLF. Following the lesion, the number of touches decreased from 144

to 49 but then increased to 108 and remained at that level. The animal increased its usage of its preferred paw (left) following the lesion. The success rate of the pellet retrieval test decreased significantly (P=0.035) at P1 compared to pre lesion, and did not recover by P3.

R601- Left paw preference

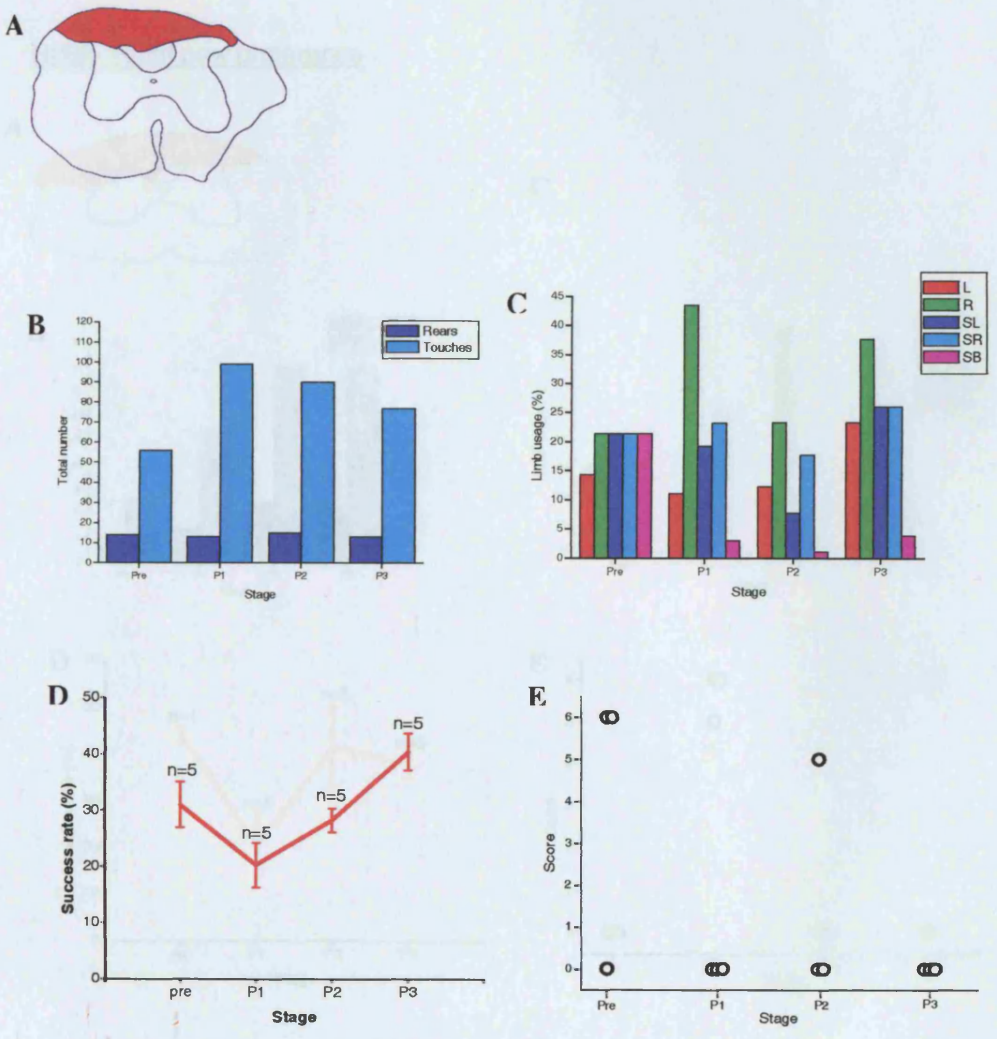


Figure 3.24 Behavioural data for R601.
A Reconstruction of lesion; **B & C** data from cylinder test; **D** data from pellet retrieval test; **E** data from sticker test. Format as figure 3.11.

The lesion to this animal was at rostral C4 and did not completely destroy the dorsal columns sparing the CST, but slightly damaged the L-DLF. The number of touches during the cylinder test remained fairly constant over the three stages and the animal used its non-preferred paw (right) more after the lesion. During the pellet retrieval test, the success rate decreased only slightly after the lesion, but then increased to a rate higher than the pre-lesion rate.

R602- Right paw preference

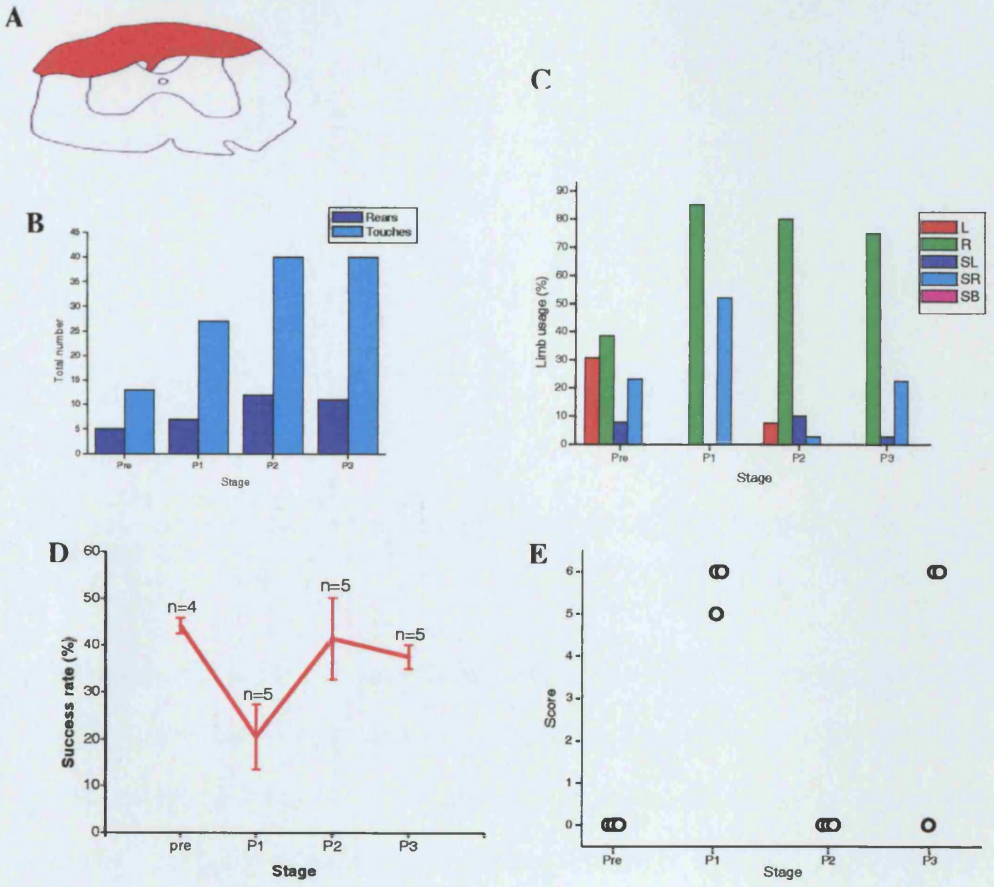


Figure 3.25 Behavioural data for R602.
A Reconstruction of lesion; **B & C** data from cylinder test; **D** data from pellet retrieval test; **E** data from sticker test. Format as figure 3.11.

The lesion spanned C4 and rostral C5 and spared some of the ventral part of the DCs, therefore most of the CST remained intact but the lesion also extended to the

L-DLF. The number of touches made during the cylinder test actually increased after the lesion. Both the left and right paws were used equally before the lesion. The left paw was not used following the lesion, neither as a single movement or a simultaneous one, which could be explained by the damage to the L-DLF (Liu *et al* 1999). At P2 and P3, the left paw was still used only minimally, and most of the contacts to the cylinder were made using the right paw. The success rate of the pellet retrieval test decreased following the lesion, although not significantly and increased at P2 almost to pre injury level.

R605- Right paw preference

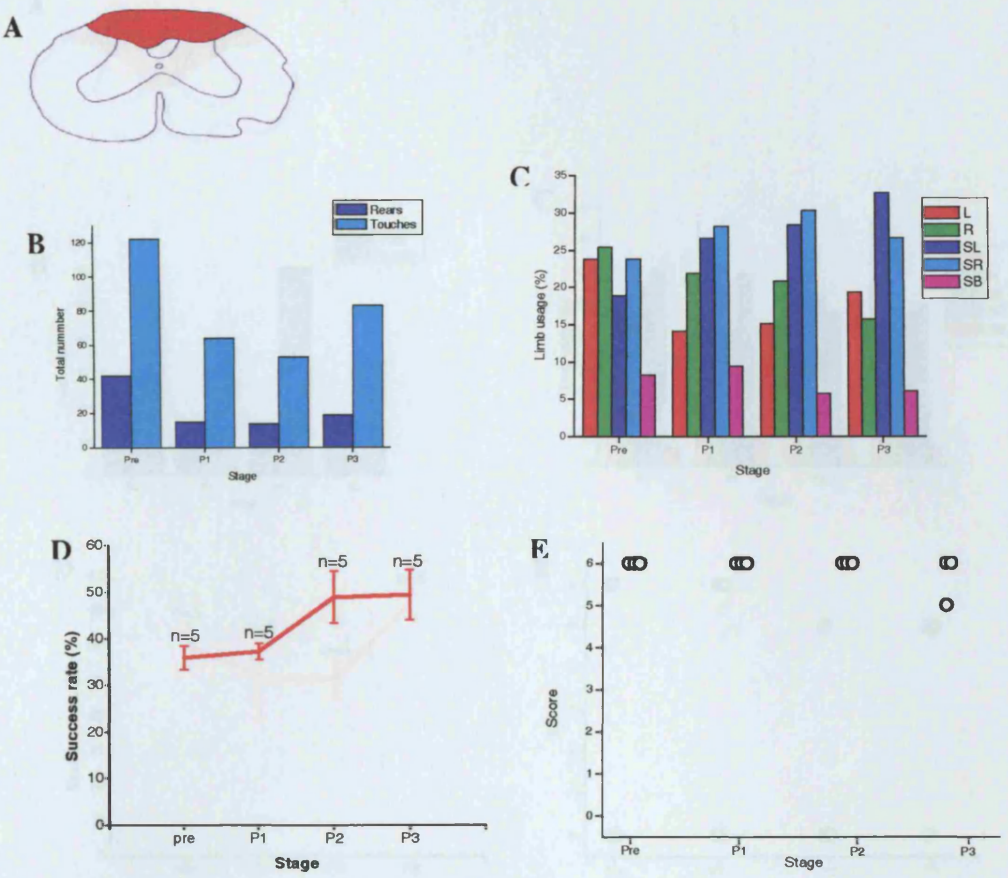


Figure 3.26 Behavioural data for R605.
A Reconstruction of lesion; **B & C** data from cylinder test; **D** data from pellet retrieval test; **E** data from sticker test. Format as figure 3.11.

The lesion spanned from caudal C4 to rostral C5 and was incomplete sparing most of the CST. The number of touches did decrease after the lesion but there was not much change is the use of the right and left paws when making contact with the cylinder. The pellet retrieval test showed no reduction in success rate, with a slight increase over the three stages.

R606- Left paw preference

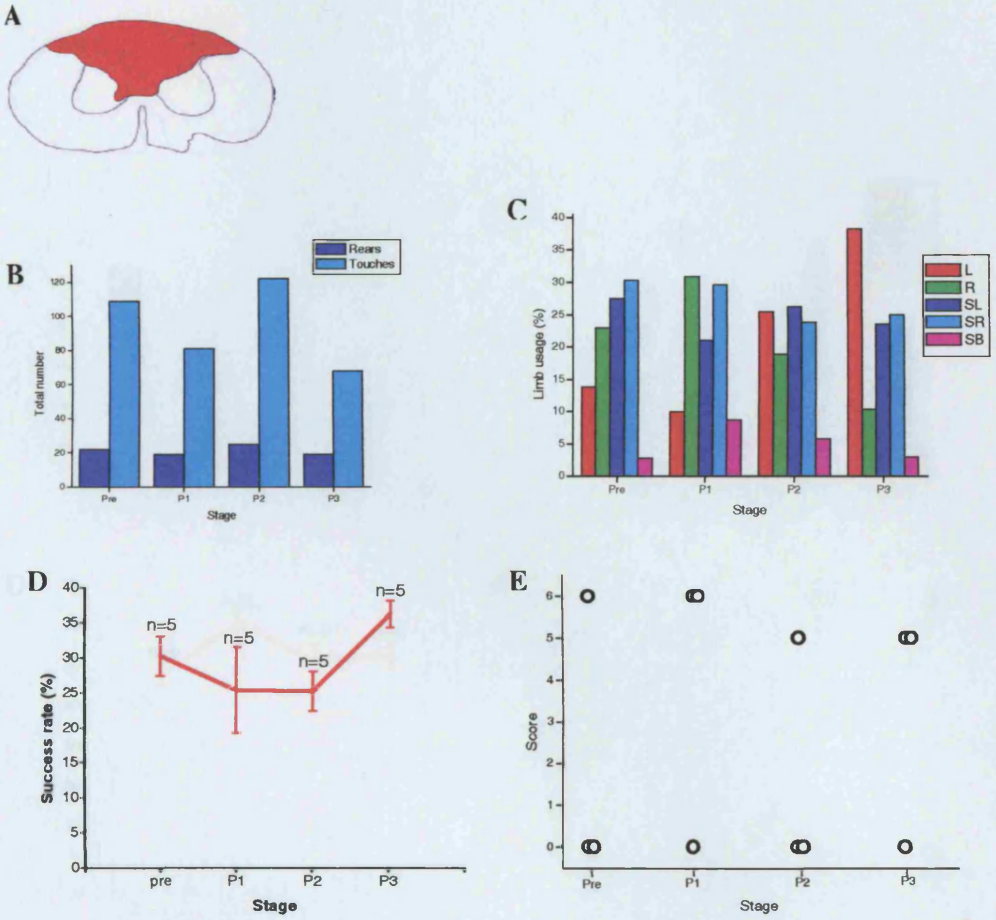


Figure 3.27 Behavioural data for R606.
A Reconstruction of lesion; **B & C** data from cylinder test; **D** data from pellet retrieval test; **E** data from sticker test. Format as figure 3.11.

The lesion spread from caudal C3 to rostral C4 and was a complete DC lesion. It extended slightly into the medial white matter and the lateral funiculi but with no damage to the DLF. The number of touches decreased slightly after the injury, and increased at P2, but decreased at P3. This animal showed a trend to using its non-preferred paw when contacting the cylinder, but reverted to using its preferred paw at P2 and P3. A non significant decrease in the success rate of the pellet retrieval test occurred, with a recovery to a rate higher than the pre-lesion rate at P3.

R607- Right paw preference

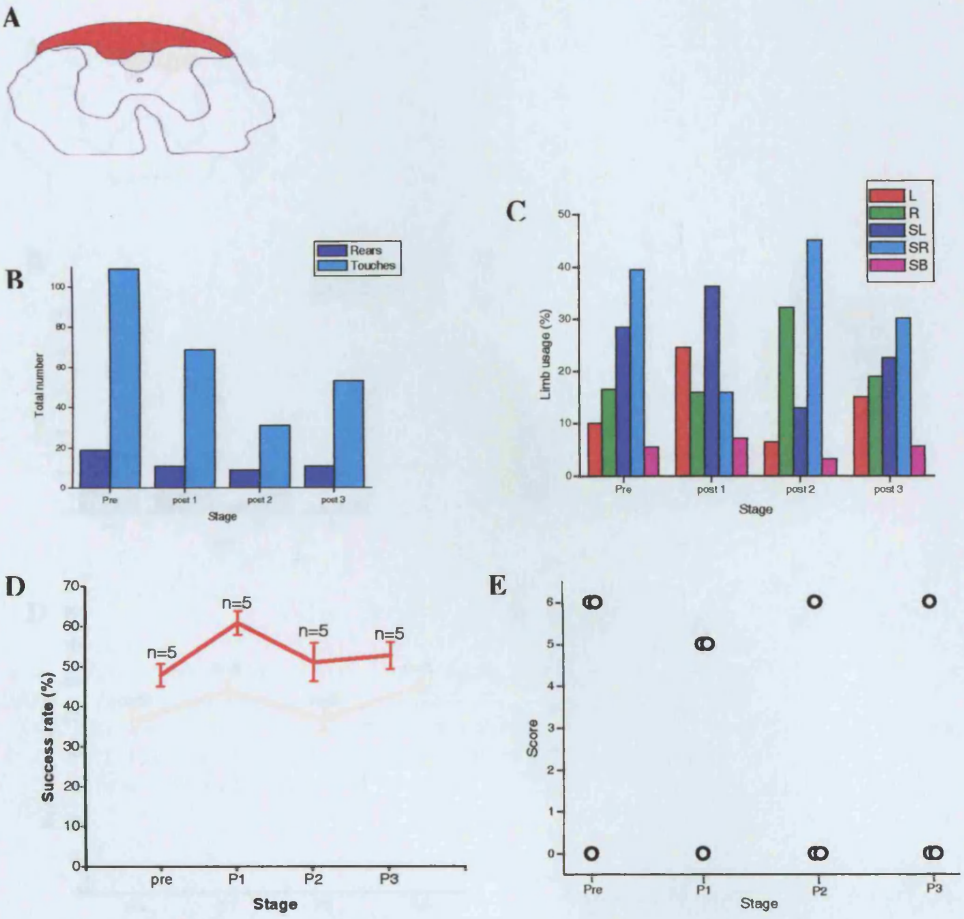


Figure 3.28 Behavioural data for R607.
A Reconstruction of lesion; **B & C** data from cylinder test; **D** data from pellet retrieval test; **E** data from sticker test. Format as figure 3.11.

The lesion spread over C3 and rostral C4, but did not completely destroy the DCs, leaving most of the CST intact. The lesion still resulted in a fall in the total number of touches made during the cylinder test. The preferred paw was used at a slightly higher rate before the injury, but the non-preferred paw was used more frequently post injury. At P2 and P3, the animal reverted to using its non-preferred paw more frequently. The pellet retrieval test showed little change in the success rate post injury.

R608- Right paw preference

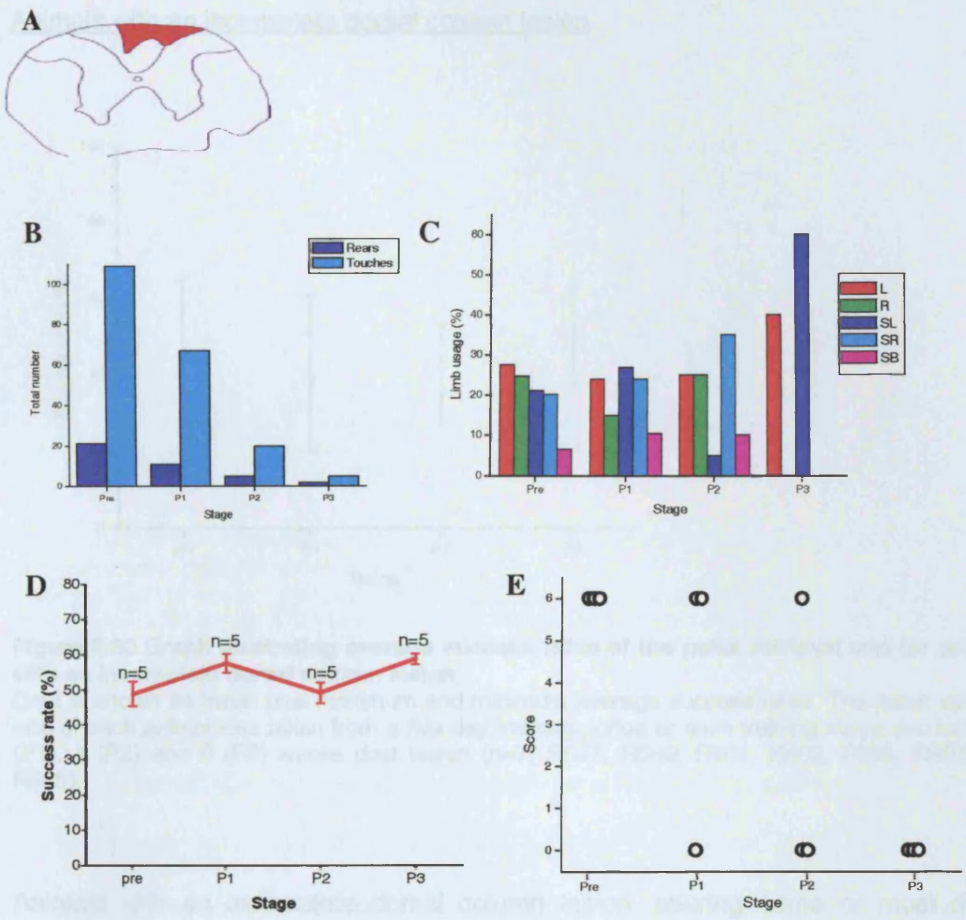


Figure 3.29 Behavioural data for R608.
A Reconstruction of lesion; **B & C** data from cylinder test; **D** data from pellet retrieval test; **E** data from sticker test. Format as figure 3.11.

The lesion to this animal spread from caudal C3 to rostral C4, but out of all animals, was the smallest lesion sparing most of the DCs and the CST. During the cylinder test, there was still a reduction in total number of touches but there was not much change in the use of both the right and left paw. Although at P3, the animal did not use it preferred (right) paw to contact the cylinder, neither as a single movement nor a simultaneous one. However, the animal only reared twice so the data for P3 cannot be compared to the pre-lesion data nor P1 and P2. There was little effect on the success rate of the reaching test.

3.3.3 Pellet retrieval group results

Animals with an incomplete dorsal column lesion

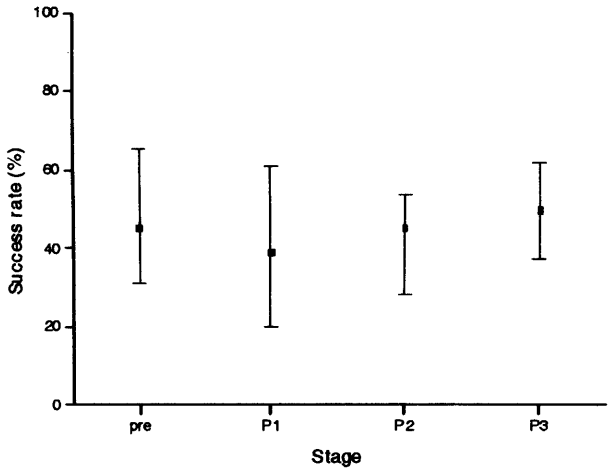


Figure 3.30 Graph illustrating average success rates of the pellet retrieval test for animals with an incomplete dorsal column lesion
Data is shown as mean plus maximum and minimum average success rates. The mean success rate of each animal was taken from a five day training period at each training stage: pre lesion, 2 (P1), 4 (P2) and 6 (P3) weeks post lesion (n=7, R547, R589, R601, R602, R605, R607, and R608).

Animals with an incomplete dorsal column lesion, sparing some or most of the corticospinal tract did not show any significant changes in the success rate of the pellet retrieval test.

Animals with a lesion specific to the dorsal columns

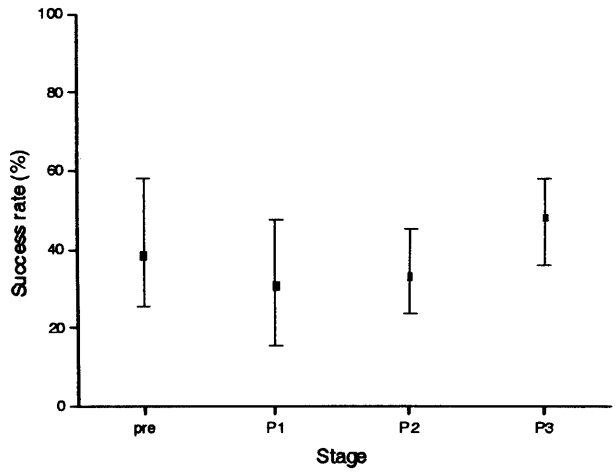


Figure 3.31 Graph illustrating average success rates of the pellet retrieval test for animals with a complete (specific) dorsal column lesion.
Data is shown as mean plus maximum and minimum average success rates. The mean success rate of each animal was taken from a five day training period at each training stage: pre lesion, 2 (P1), 4 (P2) and 6 (P3) weeks post lesion (n=4, R538, R569, R574, and R606).

It was demonstrated that four animals received a lesion to the dorsal columns, with no further damage to the vCST or the RST. In all the animals except for R569, the success rate decreased following the lesion, but returned to baseline, or above by P3. The success rate of R569 did not decrease following the lesion, but in fact increased slightly at P1. Lesions to dorsal columns have therefore no permanent effects on the success rate of the pellet retrieval test.

Animals with a lesion that extended to the v-CST and/or the DLF

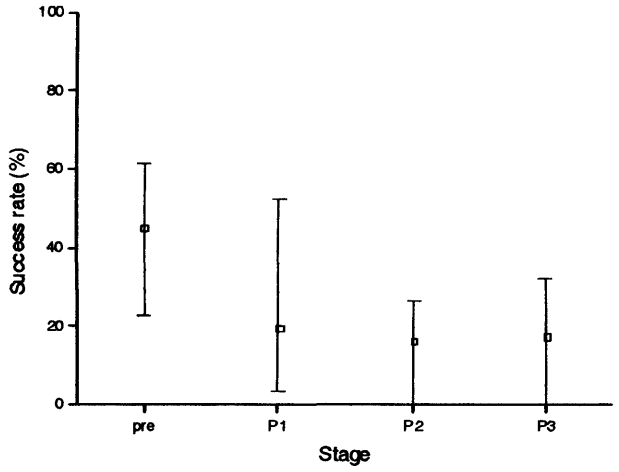


Figure 3.32 Graph illustrating average success rates of the pellet retrieval test for animals with a lesion that extended to the vCST or DLF.
Data is shown as mean plus maximum and minimum average success rates. The mean success rate of each animal was taken from a five day training period at each training stage: pre lesion, 2 (P1), 4 (P2) and 6 (P3) weeks post lesion (n=9, R522, R523, R539, R542, R543, R570, R572, R575, and R590).

Histological reconstructions revealed that nine animals had a lesion that extended beyond the dorsal columns into the v-CST or the DLF. Analysis of the pellet retrieval test demonstrated that the success rates of these animals significantly decreased after injury and remained at a low level during all the testing stages.

3.3.4 Summary

The reaching test proved to be the most discriminatory in assessing functional recovery following a bilateral lesion to the dorsal columns. Animals were trained to use their preferred paw pre lesion and post lesion allowing assessment of functional recovery. Due to the lesion being a bilateral one, the cylinder test did not provide crucial information on the recovery as both paws suffered injury and the cylinder test

would be most suitable for the assessment of the effects unilateral lesions may have on behaviour and functional recovery. R602 suffered an asymmetric lesion, which damaged the L-DLF, as a result, the animal did not use its left forepaw at P1 and P3, and only minimally at P2. During the sticker test, pre lesion and post lesion animals always achieved a score of five or six indicating that they removed the sticker in one or multiple attempts. The sticker test therefore did not discriminate between pre and post lesion behaviour. As a result, the reaching test was used to obtain information on functional recovery and the success rates were compared and correlated to the size of the lesions. Animals with an incomplete lesion to the dorsal columns showed little change in the success rate over the three testing stages. Those with a specific lesion to the dorsal columns demonstrated a reduction in the success rate of the pellet retrieval test at P1 but recovered by P3. Specific lesions to the dorsal columns therefore do not cause permanent deficits in the success rate of pellet retrieval. In those animals with a lesion that extended to the DLF or the vCST, the success rates decreased significantly and did not recover by P3. One animal (R543) with an extensive lesion extending to the left and right DLF and also the vCST was still able to reach through the slit for the pellet. Although R543 was not able to successfully retrieve pellets at P2 and P3, with the shelf 2cm away, it was able to retrieve some when the shelf was cm away. All animals displayed abnormal body movements while reaching, including dropping of the ipsilateral shoulder and abnormal rotary movements. All animals except for R608 would often drop the pellet not realising they successfully retrieved it, and in cases where the retrieval was unsuccessful would often take the paw to their mouth not realising it was empty. These two observations lead to the conclusion that animals with a significant dorsal column lesion showed signs of loss of sensation in the forepaw.

3.4 Discussion

The study of the animals' behaviour was aimed at assessing functional recovery following a cervical spinal cord injury. In this study, the main component of the corticospinal tract (CST) which is found in the ventral-most part of the dorsal columns was lesioned at cervical spinal cord level 4/5. Three end point behavioural tests were used to assess spontaneous functional recovery: the pellet retrieval test, the sticker removal test, and the cylinder test.

Twenty animals were used in this study and histological evaluation of the lesion site showed variation in lesion extent. In order to correlate the degree of lesion with the spontaneous functional recovery, animals were divided into three groups according to the extent of the lesion. Seven animals received an incomplete lesion to the dorsal columns, four sustained complete dorsal column lesions with no damage to the rubrospinal tract or the ventral CST, and nine received complete dorsal column lesions, but also extended to the vCST or the RST or both.

The results demonstrate that if the dorsal columns were destroyed, with no further damage to the rubrospinal tract or the ventral CST, there was little effect on the success rate of the pellet retrieval test. The cylinder and sticker removal tests did not provide valuable information on the recovery. Following the lesion, all animals except R608 displayed abnormal body movements while reaching for the food pellet and showed signs of loss of sensation in the forepaw used to retrieve the pellet. Because the pellet retrieval test provided the most discriminative information on spontaneous functional recovery, deficits and changes in behaviour as assessed during this test are discussed, including the change in the reaching strategy, grasping abnormalities, and targeting deficits. There is minimal discussion of the sticker removal and cylinder test. This is followed by discussion of the animal groups. To allow easiness of

correlating post-lesion behaviours to damage of spinal pathways, lesion groups are divided into two groups:

1. Dorsal column lesion group: animals with both incomplete and complete dorsal column lesions.
2. Combined lesion group: animals with a lesion extending to the dorsolateral spinal cord and/or ventral/ventrolateral spinal cord.

Before discussing the results, I would like to make it clear that changing the pellet size during training and testing of the pellet retrieval test had no effect on the results. Initially pellets weighing between 40 and 100mg were used during the pellet retrieval test. The pellets were carefully selected in order to maintain a similar size and weight during the training and testing period. During the later stages of this study, precision pellets were used weighing 45mg. Although the pellet size was changed, there was no evidence that the success rate was affected. It has previously been reported that success rates are affected only if the pellet size was significantly increased (Metz & Whishaw, 2000). In their study, Metz and Whishaw demonstrated that success rate of the pellet retrieval test was maintained with pellets weighing 20-190g, and decreased when pellet size was increased to 300g. This is reflected in the movement rating, where animals were clearly unable to change their movements to compensate for the large increase in pellet size.

3.4.1 Targeting and grasping deficits

Dorsal column lesions cause deafferentation of the cuneate, external cuneate, and internal basilar nuclei, which could lead to the loss of forelimb prioceptive influences. Lesions to the CST have previously been reported to be associated with targeting and grasping deficits during the pellet retrieval test. Following pyramidotomy in rats,

target deficits were more prominent when the damage extended to the medial lemniscus (Castro, 1972). This was supported by Schrimsher and Reier's (1993) experiments, where they showed that dorsal column lesioned animals exhibited targeting deficits. A study aiming to assess whether peripheral input from a moving forelimb is essential for target reaching in rats following a dorsal rhizotomy, demonstrated that peripheral input from a moving forelimb is necessary for the effective performance of grasping (Saling *et al.*, 1992). The targeting deficit demonstrated during reaching for a food pellet following a dorsal column lesion suggests that somatosensory feedback is required for grasping. All animals within the present study (except for R608) demonstrated targeting and grasping deficits during the pellet retrieval test. Animals often found it difficult to reach diagonally for the pellet placed in the indentation contralateral to the preferred paw and would therefore reach straight ahead and by doing so score a higher number of unsuccessful reaches. Many animals in the present study would also simply reach too high or too low, or even too far. Although these observations where not quantified, it was clear that these impairments were present in all animals with a significant lesion to the dorsal columns. Therefore the results of previous studies and the current study provide evidence that loss of sensory input leads to target impairment.

Grasping impairments during the pellet retrieval test have frequently been described. Schrimsher and Reier (1993) demonstrated that following a lesion to the dorsal columns at cervical level 5, animals did not show a significant reduction in the success rate of the pellet retrieval test up to 4 weeks post injury. However, the animals did show limited lateral spread of the digits as the paw approached the pellet, but extension of the digits was present. Also, lateral spread of the digits was not maintained during the digit flexion phase of the grasp. In the present study, similar deficits were seen. During the pellet retrieval test, the animals easily reached for the food pellet but found it difficult to achieve the grasp, so that when the paw

reached the pellet, it is was not always closed into a grasp, resulting in the animal knocking the pellet off the shelf and scoring an unsuccessful reach.

When an animal grasps the food pellet, it uses an arpeggio movement, where the paw pronates from digit 5, which is the outer digit to digit 2 preparing for the grasp (Whishaw & Gorny, 1994). This movement is thought to be mediated by the red nucleus (Whishaw & Gorny, 1996;Whishaw *et al.*, 1998), and is also thought to be associated with the corticospinal tract as demonstrated in studies of pyramidal lesions (Whishaw & Metz, 2002) and dorsal column lesions (Schrimsher & Reier, 1993). Schrimsher and Reier (1993) reported that changes in the grasping behaviour could be due to the loss of primary afferent axons following a dorsal column lesion. In our animals with a lesion to the dorsal columns, the loss of the arpeggio movement was clearly demonstrated in all animals here with a complete lesion to the dorsal columns. It was difficult to confirm whether those with an incomplete dorsal column lesion demonstrated the arpeggio. The grasping of the pellet was very precise, but because of the speed of the video recording, it was not clear whether this movement was an arpeggio one, but it most likely was or was very similar to it. Either way, it was not the clear "scooping" movement carried out by the extensively lesioned animals. The absence of forelimb peripheral input also produces a marked decrease on the grasping success of the rat during the pellet retrieval test (Saling *et al.*, 1992). It is therefore possible that the grasping deficit observed following a dorsal column lesion could be the result of combined damage to the CST and ascending sensory fibres found in the dorsal columns.

3.4.2 Alternative reaching behaviour following a lesion to the dorsal columns

Pre-lesion, all animals successfully reached through a slit in the training box and retrieved food pellets placed on a shelf using "normal" body movements. These movements, as previously described (Whishaw & Pellis, 1990;Whishaw & Gorny, 1994) were as follows: the animal oriented its head towards the target (pellet) and the snout was inserted through the slot. The limb was lifted, digits were closed and the animal aimed for the pellet. As it advanced the limb, the digits opened and the pellet was grasped using the arpeggio movement. The paw was supinated to 90° so that the animal could withdraw the paw through the slit of the box, and the paw was then supinated again, this time to 180°, so that the paw faced the mouth. The animal then sat back, brought the paw to the mouth as the head was lowered and the pellet was consumed. Following a lesion to the dorsal columns, the animals were still able to successfully retrieve the food pellets but altered reaching movements were used. The body was rotated as the animal aimed for the pellet and the ipsilateral shoulder was dropped. The animals rapidly grasped the pellet with a scooping motion rather than the described arpeggio movement. Supination 1 & 2 was also absent, and as a consequence, the animals brought their body and head closer to the pellet in order to consume it. The absence of independent supination was also described by Mckenna and Whishaw (1999). Therefore, although dorsal column lesioned animals were able to successfully retrieve pellets, compensatory movements were used. Similar observations were also previously demonstrated by Whishaw and colleagues. In their study, they assessed the importance of the ascending dorsal column pathways in relation to skilled reaching in the rat (McKenna & Whishaw, 1999). The lesion performed was a unilateral lesion cutting the ascending component of the dorsal columns, sparing the CST. This lesion was performed at C2, and therefore none of

the motoneurons affecting forelimb muscles were affected. Their results demonstrated that although pellet retrieval was affected at the early stages post injury, it recovered by day 7 post injury. However, kinematic analysis revealed that dorsal column injured animals retrieved food pellets successfully by adjusting body and digit movements. Previous studies have demonstrated that abnormal behavioural movements are present after a lesion to the DLF, with the dorsal columns spared (McKenna & Whishaw, 1999). This could be attributed to the loss of the lateral component of the CST, or possibly other lateral pathways.

The development of a different reaching strategy following a lesion to the dorsal columns implies that somatosensation is important in forelimb function.

3.4.3 Loss of sensation in the rat forepaw following a dorsal column lesion

Assessment of behaviour during the pellet retrieval test indicated possible loss of sensation in the rats' forepaw. The animals often successfully retrieved the food pellet, but subsequently dropped it; apparently not realising that it was in their forepaw. In addition to this, the animals often missed the pellet, but then went on to take their forepaw to their mouth in order to consume the pellet. Both these behaviours lead to the conclusion that animals with a lesion to the dorsal columns have lost sensation in the forepaws. Although animals are able to successfully retrieve food pellets, this loss of sensation greatly affects the success rate of the pellet retrieval test. This could be attributed to the loss of ascending sensory pathways in the dorsal columns including the dorsal column medial lemniscal

pathway and the post-synaptic dorsal column pathway. Axons from these pathways that ascend from the lumbar spinal cord are found within the fasciculus gracilis, those ascending from the cervical spinal cord are found within the fasciculus cuneatus (Giesler, Jr. *et al.*, 1984)

Ballermann and colleagues reported that animals with a lesion to the dorsal columns could not discriminate between a food item and a tactually distinctive non food item (Ballermann *et al.*, 2001). They concluded that the dorsal columns may play an important role in on-line tactile discriminations, or haptic actions (hapsis is the sense of touch during the grasping movement). In their study, they argued, that although the lesion destroyed both sensory and motor components of the dorsal columns, the deficit seen is not a motor deficit. Their reasoning for this was that the dorsal column lesioned animals performed at control levels on other tests of sensory and motor function. The injured rats were as successful as the control when reaching for a single piece of pasta and also exerted similar forces when breaking off pasta as that of the controls. They also argued that the sensory deficit they observed seemed to be selective to the grasping action. The question to be addressed next is whether this deficit is due to impairment in sensory detection or simply a motor deficit. In their study, the animals were able to detect a sticker placed on the forelimb, and also didn't show deficits during the cylinder test, where the forepaws are used to support the animal's weight and explore the sides of the cylinder while rearing. These results are consistent with the results of the sticker and cylinder tests used in this study.

The behaviour of the animal taking its paw to its mouth after missing the food pellet is reflected in a study that examined sensory deficits during motor performance following an injury to the primary motor cortex in the monkey. Following the lesion, the animal examined its paw after reaching for a food item, although the food item had not been grasped (Nudo *et al.*, 2000). This sensory impairment was present

straight after the injury, peaked at 5 days but had returned to normal levels by day 11. Schrimsher and Reier reported that following a lesion to the dorsal columns (destroying sensory and motor components), rats developed a chronic "paw checking" behaviour consisting of the animal retracting the paw and sniffing it for the presence of a pellet (Schrimsher & Reier, 1993). The persistent behaviour of "paw checking" observed in this study and others could be due to the loss of tactile feedback, as the dorsal columns are the main route for transmission of tactile perception.

A recent study demonstrated that destruction of the ascending part of the dorsal columns (C4), but not the DLF resulted in the loss of somatosensory-evoked potentials evoked by electrical stimulation of the forepaw (Onifer *et al.*, 2005). It was also noticed that the time taken for an animal to notice the sticker on the forepaw was prolonged by one week following a lesion to the dorsal columns. These results provide evidence that the sensory pathways found in the dorsal columns are important for cutaneous sensation. However, animals with a dorsal hemisection at C4 showed improved results, where the time taken to notice the sticker became less over the four week period. Onifer and colleagues attributed this improved sensory function to a learning or training of the test. Their reasoning for this was that when the animals were tested only once at 4 weeks post injury, they did not show reduced time taken to notice the sticker in their forepaw.

From the above studies, it is clear that the ascending dorsal column sensory projections are important in skilled target reaching of the rat.

3.4.4 Lesion groups

Lesion to dorsal column with no damage to the ventral CST and/or the DLF

Seven animals received a lesion to the dorsal columns that did not completely destroy the main component of the corticospinal tract (R547, R589, R601, R602, R605, R607, and R608). In all animals but R547 and R589 there was no significant difference in the success rate of the pellet retrieval test post-lesion as compared to pre-lesion. This could be explained by the small amount of CST spared. The success rate recovered almost to the pre-lesion level by the third testing stage post lesion (P3). R589 did not train very well pre-lesion or post-lesion, and therefore the success rates may not be representative of the recovery. The success rate of R601 & R602 fell after the lesion, but recovered in both animals by P2. Histological reconstructions of the lesion demonstrated that most of the CST was spared in both animals, but the L-DLF of both animals and the R-DLF of R602 was damaged resulting in damage to the rubrospinal tract ispilateral to the paw used during reaching (left for R601, and right for R602). This could explain the decrease in performance at P1 as the rubrospinal tract has been shown to be important in skilled reaching of the rat. R605, R607, and R608 received a lesion that spared probably all the CST in the dorsal columns and as a result the success rate of the pellet retrieval test was not affected following the lesion.

Four animals received a lesion that destroyed all of the dorsal columns (R538, R569, R574, and R606). The success rate of pellet retrieval test in these animals, except for R569, showed a reduction (non significant) following the lesion (P1), which recovered over time. R569 showed very little change in the success rate, which actually increased during P1.

In agreement with our results, Schrimsher and Reier (1993) also reported that animals with a lesion to the dorsal columns did not show a significant reduction in the pellet retrieval test at any post-lesion stage (up to 4 weeks post-lesion).

The results of the present study therefore demonstrate that lesions to the dorsal columns have a persistent effect on the skilled target reaching of the rat even when the corticospinal tract is not completely severed. These deficits in the reaching pattern are still present when damage to the CST is minimal as was demonstrated in R607 and could therefore be attributed to the loss of sensory afferents. The afferent input from the dorsal columns must therefore play an important role in rubral and cortical motor control in the rat as it has been previously described in the cat (Rathelot & Padel, 1997). However, afferent information from the forelimb can reach the brain through other pathways such as spinothalamic and spinoreticular tracts which could in turn compensate for the loss of input via the dorsal columns.

Lesion to dorsal column with damage to the ventral spinal cord and/or the DLF

Histological reconstructions demonstrated that nine animals received lesions that not only completely destroyed the dorsal columns, but also extended to the ventral funiculus, ventrolateral funiculus, and/or the dorsolateral funiculus (R522, R523, R539, R542, R543, R570, R572, R575, and R590).

Following the lesion, the success rate of pellet retrieval test decreased significantly in all animals except for R572 and R575. The reaching success did not return to pre-lesion levels in any animal.

Some animals in this lesion group would have undergone loss of motoneurons in the ventral horn (R542, R543, and R570). This would subsequently lead to major impairment of the forelimbs due to the loss of motoneurons for the spinodeltoideus, biceps, extensor pollicus longus, and the extensor carpi radialis muscles (McKenna *et al.*, 2000). The affect this has on the muscles would most likely result in impairments in shoulder and elbow flexion and carpus extension. This is reflected in the difficulty these animals had in extending their forelimbs and getting the limb through the slit of the pellet retrieval box where animals would often hit the side of the slit while trying to reach for a pellet.

Extension to DLF

Lesions that extended beyond the dorsal columns and into the DLF resulted in permanent deficits during the pellet retrieval test, resulting in a decrease in the success rate which did not recover over time. Damage to the DLF causes damage to a number of descending pathways including the rubrospinal tract and the lateral component of the CST. The DLF also contains a small lateral component of the reticulospinal tract which is thought to be involved in locomotion (Loy *et al.*, 2002).

It would be easy to conclude from these results that a combined lesion of the main component of the CST and the DLF leads to irreversible deficits during target reaching. However, previous studies have demonstrated that animals with combined lesions of the DLF and the dorsal columns, but with the dorsal CST intact do not recover to pre-lesion levels during the pellet retrieval test (McKenna & Whishaw, 1999). We can speculate that the loss of input from the CST via the dorsal columns is probably sufficient to cause permanent behavioural deficits when combined with a lesion to the DLF.

Schrimsher and Reier (1993) reported that following a lesion to the DLF ipsilateral to the preferred paw, animals showed a significant reduction in the success rate of the pellet retrieval test at all weeks post lesion. The animals were able to target the pellet but were unable to execute the grasp due to the inability of closing the digits around the pellet. However, the findings of a study by Whishaw and colleagues did not agree with this. Unilateral ibotenic acid lesions of the red nucleus did not affect the success rate of the pellet retrieval test of the ipsilateral nor the contralateral forelimb, and the animals were also able to flex the digits of the impaired limb (Whishaw *et al.*, 1990;Whishaw *et al.*, 1998). In their study, Whishaw and colleagues showed that when a red nucleus lesion was added to a previous motor cortex lesion, the total number of reaches was not affected, nor was the success rate. However, there was an affect on limb accuracy and paw opening during grasping. From this they concluded that the red nucleus may not be essential for the ballistic component of reaching but may contribute to the fine motor control. The difference between these two studies is the paradigm used for assessing pellet retrieval. Whishaw's animals were required to reach across a 0.5cm gap and grasp pellets from a pellet filled container, and Schrimsher's animals were required to reach across a 3.0cm gap and reach for the pellet placed on a shelf. Schrimsher states that the impairment was most obvious when the rats tried to flex their digits against the resistance offered by the shelf, and therefore would not be prominent if the pellets were grasped from a container. A lesion to the DLF results in damage to other spinal pathways which could account for the difference in results obtained from these two studies. Spinocerebellar pathways and postsynaptic ascending sensory pathways run in the DLF. These pathways could play a role in the grasping behaviour of the rat during reaching. Grasping impairments were also reported following a rhizotomy of forelimb dorsal roots which could be attributed to the loss of afferent inputs relayed through the dorsolateral spinal pathways (Saling *et al.*, 1992).

Unilateral cervical spinal cord hemisections (C5) resulted in a lower success rate of pellet retrieval compared to control (Anderson *et al.*, 2005). These rats had impaired grasping ability and impaired pronation and supination of the forelimb. However, if the hemisection was incomplete, animals were able to perform a few "normal" reaches (20%) with normal grasps, and normal supination and retraction.

Extension to the ventral spinal cord

In the present study, animals with lesion pathology extending into the ventral or ventrolateral funiculus did not recover to pre-injury levels during the pellet retrieval test. Deficits persisted during all post lesion stages including target and grasp deficits.

It was previously demonstrated that sparing of both the ipsilateral dorsal and ventral funiculi results in improved performance during reaching following a contusion injury at cervical level 4/5 (Schrimsher & Reier, 1992). By 1 week post injury, animals were able to maintain a quadruple stance. Interestingly, animals that regained reaching had nearly complete destruction of the main corticospinal spinal tract with preservation of the fasiculus cuneatus and gracilis.

Spontaneous recovery of forelimb reaching following a bilateral lesion to the dorsal columns at cervical spinal cord level 2 was prevented when the ventral component of the CST was lesioned (Weidner *et al.*, 2001). It was also demonstrated in this same study that sprouting of the ventral component of the CST occurred following a lesion to the dorsal columns which coincided with spontaneous functional recovery at 4 weeks post lesion. Sprouting of ventral corticospinal fibres occurred onto motoneurons located medially in the ventral grey matter.

It has been reported that sparing as little as 1% of the CST in the rat following a unilateral lesion of the main CST results in improved reaching in the forelimb ipsilateral to the injury (Keyvan-Fouladi *et al.*, 2003). However, these results cannot be directly compared to the results of the present study due to the difference in the training and testing paradigm. In the above study, the animals were allowed to use either the ipsi- or contralateral forepaw to grasp the pellet, and the number of successes was then scored. This is different to the present study, where animals were "forced" to use only the "preferred" paw.

A study into forelimb performance following selected spinal cord lesions at C5, demonstrated that following a ventrolateral funiculi lesion, the grasping behaviour was not affected, but animals showed substantial reaching hypometria at 1 week post lesion, which reduced with time. The hypometria described involved impaired shoulder flexion and elbow extension that may be caused by a loss of reticulospinal and/or vestibulospinal input (Schrimsher & Reier, 1993). In their study, one animal that sustained a lesion that unilaterally extended to the ventrolateral white matter and the ventral horn, showed a deficit in extension of the ipsilateral forelimb while reaching. This deficit was so severe, that an operationally defined reaching attempt could not be made. In their study, the reaching attempt was defined as the movement that resulted in the rat contacting the recessed tray with its forelimb or extension of the forelimb over the upper surface of the tray. However, in the present study, and in those animals with a lesion that extended to the ventral white matter and ventral horn, animals were still able to reach through the slit and retrieve the pellet (R542, R570). One particular animal, R543 that sustained an extensive lesion destroying the dorsal columns, dorsal horns, ventral white matter, with only partial sparing of the ventral horn and DLF, was still able to reach through the slit, but did not score any successful retrieval when the shelf was 2cm away. However, when the shelf was placed 1cm away, the animal was capable of successfully retrieving a few pellets. In

those with a ventrolateral lesion, animals showed a significant reduction in the success rate of the pellet retrieval test at 1 week post lesion but recovered by week 2.

In addition to the above mentioned deficits, animals with a lesion extending to the ventral spinal cord displayed balance deficits, and would sometimes fall over while grooming or during vertical exploration in the cylinder test. This can be explained by previous observations by Webb and Muir where they assessed sensorimotor recovery in rats following a unilateral ventrolateral lesion (C4). They reported that animals showed impaired forelimb and hindlimb usage immediately after the spinal cord lesion (Webb & Muir, 2004). Injured animals fell between rungs of a ladder more frequently than that before surgery. Following injury, and during the cylinder test, animals used the limb ispilateral to the injury less, and both limbs together more compared to limb usage pre-injury. During locomotion, injured animals bore less weight on the fore- and hindlimb ipsilateral to the lesion. However, injured animals recovered to near normal levels after five and half weeks post lesion. Although, in the case of locomotion, animals did continue to bear less weight on the hindlimb ipsilateral to the injury compared to the contralateral one. It could be therefore concluded that pathways in the ventrolateral funiculus can be sub-served by remaining ones following an injury (Webb & Muir, 2004).

The vestibulospinal tract descends predominantly ipsilaterally within the ventral and ventrolateral funiculus of the rat. Following a ventrolateral lesion, this tract would be damaged and could cause motor impairments. Work by Webb and Muir implies that this tract is important in extensor muscle activation and flexor muscle inhibition. Impairments in these muscles could lead to an increased number of foot falls during ladder crossing, and also reduced usage of the limb ipsilateral to the injury during vertical exploration. However, because these deficits are not permanent, the

pathways found in the ventrolateral funiculus are not absolutely necessary for skilled locomotion or for forelimb usage during vertical exploration.

So, if the main aspect of the corticospinal tract is lesioned, functional recovery will occur which has been attributed to plasticity from the ventral CST. The ascending projections of the dorsal columns also play an important role in skilled reaching in the rat.

It could be concluded from these results that behavioural functional recovery is dependent on the amount of CST fibres spared in the dorsal columns.

3.4.5 Behavioural tests and their suitability

Cylinder test

The cylinder test is a non skilled test that does not require training in the rat. When the rat is placed in the cylinder, the animal rears, using its forepaws to balance and support its weight while exploring the walls of the cylinder. The main parameter measured in this study was paw usage for support while rearing.

In this study, the cylinder test did not provide crucial information on the functional recovery of the animals following a bilateral dorsal column lesion. The cylinder test is most suitable for the assessment of chronic limb use following a unilateral lesion as this would allow the comparison of the injured limb versus the uninjured one. In the present study, one animal sustained an asymmetric lesion (R602), consistent with this; it demonstrated asymmetry in forelimb use following the injury.

It has previously been reported that animals with a severe cervical injury (C5) sparing little grey and white matter do not, or maybe cannot rear against a vertical surface (Pearse *et al.*, 2005). In contrast to this, one animal in this study (R543) sustained an

extensive lesion, also at C4/C5, and was able to rear and support its weight using its forelimbs.

Previous studies reported that the rubrospinal tract is important in producing the behaviour used in the cylinder. These studies demonstrated that when the rubrospinal tract is injured at the cervical level, rats use the forelimb ipsilateral to the lesion significantly less than the contralateral limb (Liu *et al.*, 1999;Webb & Muir, 2003).

As to whether the dorsal columns play an important role in this behaviour is unclear. Two studies show conflicting results in regards to the use of forelimbs during rearing following a unilateral lesion to the dorsal columns, with sparing of the main component of the corticospinal tract (Ballermann *et al.*, 2001;Webb & Muir, 2003). Ballermann and colleagues demonstrated that following a unilateral lesion to the dorsal columns at C1, there was no significant difference in paw usage between the injured and control groups. Webb's study used animals with a unilateral lesion at C4 showed that injured animals used their injured paw significantly less than the uninjured one. The lesions in both Ballermann's and Webb's studies spared all or most of the CST in the dorsal columns. From the present study, it cannot be fully ascertained whether the dorsal columns play a role in the cylinder behaviour as the lesion was a bilateral one and there was no difference in limb usage after the lesion. In general, animals reared less and the number of contacts made with the cylinder was also less. However, there were no significant differences in limb usage before and after the lesion.

Sticker removal test

The removal of a sticker placed on the bridge of the animal's nose requires a full range of motion and some grasping ability. It has previously been described that animals with an over-hemisection of the spinal cord at cervical level 3, did not achieve a score higher than 2 in the sticker removal rating scale (Diener & Bregman, 1998). This is in contrast to the results obtained in this study, where all animals achieved a score of 5 or 6 at all stages post lesion. Following a contusion injury at cervical level 4/5, animals also showed a significant reduction in sticker removal, and by 16 weeks post contusion, animals were able to reach as high as the sticker and managed to contact the head (but not the sticker) and some were actually able to contact the sticker, but in either case, sticker removal was not achieved (Schrimsher & Reier, 1992). Therefore, previous studies have successfully used the sticker removal test to assess functional recovery but this was not the case in this one. A contusion injury, or an over hemisection would cause damage to a number of pathways in the spinal cord. In the present study, even the most severely lesioned animal (R543) was able to remove the sticker. In this animal, there was partial preservation of the DLF and therefore may be concluded that survival of the DLF (perhaps because of the RST) could enable the animal to remove the sticker from the head.

All animals in this study were able to remove this sticker, which was done as part of the grooming movements. Because the sticker was not removed by means of a specific grasping movement, and it would be difficult to analyse the precise detail of the grooming movements without high speed video recording, this test is not suitable for the assessment of behavioural functional recovery following a lesion to the dorsal columns, nor it seems for combined lesions of the dorsal columns and DLF.

Therefore, although the sticker removal test provided crucial information on the recovery in other labs, it clearly did not in ours, as there was no difference pre- and post-lesion. Detailed analysis of the grasping behaviour may provide better information but this test is meant to be an easy to perform, easy to score test. If more experiments were to be carried out at a later stage within this lab, the sticker would be best placed on the forepaw. In this way it would probably provide more information on the loss of sensation in the forepaw as was demonstrated in the pellet retrieval test.

Pellet retrieval test

Skilled reaching is a sensorimotor behaviour used in many animal species and also humans (Iwaniuk & Whishaw, 2000). This form of behaviour requires integration of sensory and motor processes. The red nucleus, corticospinal tract, cerebral cortex, and basal ganglia are important in skilled reaching. This test is easy to perform and it is relatively easy to train the animals.

Here, the pellet retrieval test was most discriminatory in assessing functional recovery following a cervical spinal cord injury. Although this test might appear to be primarily a motor test, behavioural deficits were seen which imply sensory impairments.

3.4.6 Alternative behavioural tests

The three tests used in this study are classified as end-point measures. The advantage of end-point measures is that they are objective, simple to score, and once the animal is trained, quick to perform. However, a major disadvantage is that

they do not provide information on how the task is carried out. For example, if the animal compensates following a spinal cord injury, that is to say they do not use their original movements when performing the task (McKenna & Whishaw, 1999), this could mislead the experimenter into mistakenly scoring a recovery.

Another disadvantage of end-point measures is that training animals could result in them learning the task and in this way improve their performance. Measurements taken may not necessary reflect recovery due to regeneration or plasticity but could quite possibly simply reflect the "learning" of the task.

Continuous kinematic measures could be carried out, for example measuring joint angles or limb segment positions during the cylinder and pellet retrieval test (Muir & Webb, 2000). The advantage of this kind of assessment is that unlike end-point measures, it provides detailed information on the way the behaviour is carried out.

The pellet retrieval test used in this study is a skilled test that requires the animal to be acquainted with the apparatus and trained for this test. Other tests could be used that do not require skilled behaviour, and therefore do not require the animals to be trained. Examples of these include the grip strength test and the paw placing test. The grip strength test that has been shown to provide reliable information on the recovery of forelimb function following a cervical spinal cord injury in the rat (Anderson *et al.*, 2005).

Kinetic measures are valuable in assessing locomotor deficits and involve sensitive and non-invasive techniques. Ground reaction forces, which are the forces exerted through the limb onto the ground, gives an indication on how much support each limb provides, and allows comparison of injured and uninjured limbs following a spinal cord injury. For example, force measurements were used to assess sensory

discrimination during skilled reaching in rats (Ballermann *et al.*, 2000). An advantage of introducing kinetic measurements to assess behaviour following experimental spinal cord injury is that it provides information on compensation patterns used by the animal following an injury.

Introducing kinetic measures to this study would have been very beneficial, as it was clear that following a dorsal column lesion, animals used compensatory movements during the pellet retrieval test. Force measurements would have provided additional information on the way this compensation was carried out.
In addition to these non-invasive behavioural measurements, electrophysiological assessments can also be used to analyse the behavioural changes following an injury to the spinal cord. For example, recordings could be made from muscles during movement. A main advantage of this method would the ability to acquire precise and specific information obtained from direct muscle activation.

Therefore, to obtain the maximum and most accurate information, a combination of tests must be used to assess the various motor and sensory deficits caused by spinal cord injury in the rat. As it turned out, the additional information given from the cylinder and sticker tests did not add much to the pellet retrieval test because the lesions here were bilateral.

3.4.7 Conclusion

It is clear that the success rate of the pellet retrieval test is dependent on the size of the lesion, and consequently which pathways are destroyed. Animals with a lesion that extended to the DLF and/or the ventral spinal cord did not recover to pre-lesion rates. All animals with significant injury whether the CST is lost or not, demonstrated

abnormal body movements, and showed a loss of the arpeggio, supination 1, and supination 2 during the pellet retrieval test. In addition to this, these animals displayed behaviours leading to the conclusion that loss of sensation in the paw had occurred. Therefore, ascending pathways in the dorsal columns contribute to skilled reaching, and is strongly suggested that somatosensory feedback is required for this. Animals with complete dorsal column lesions were able to return to their pre-lesion success rates by the fourth week post lesion (P2). From these results, it is clear that although the main component of the corticospinal tract is important in skilled reaching, the loss of it does not result in decreased success rates. If the rubrospinal tract or even the ventral corticospinal tract is spared, recovery of skilled reaching can occur, although reaches are carried out using an "alternative strategy".

3.4.8 Contribution of electrophysiological findings to the details of lesion extent

Cord dorsum and field potential recordings indicated that the anatomical descriptions of the lesions were not completely accurate, as the anatomical definitions of lesion extent provided no information on the physiological propertied of RST fibres in the DLF. Two animals with a lesion that anatomically extended to the DLF (R539 & R590) showed normal potentials as recorded from the cord dorsum. However, a focus of positivity was seen in the intraspinal record of the DLF in animal R590, which may represent damaged DLF fibres. Surprisingly, "normal" intraspinal maps of field potentials were produced in these two animals, as was for animal R543 with a large lesion sparing very little DLF. It is obvious here, that although anatomically damage of the DLF was defined, the RST is clearly partially preserved physiologically as demonstrated by the "normal" intraspinal maps.

Likewise, when anatomical definition of lesion extent was confined to the dorsal columns, with no lesion pathology extending to the DLF, recordings from the cord dorsum indicated otherwise. Animals R538 and R601 both showed that the cord potential ipsilateral to the stimulation site in midbrain was dominated by positivity, which indicates that fibres in the DLF may have been injured. The physiological damage to the ipsilateral spinal cord would not have had an effect on the success rates of pellet retrieval, as the preferred limb was on the contralateral side, which physiologically appeared to be normal.

Anatomical descriptions of lesion extent do not provide vital information on the physiological properties of the fibres, which is critical when associating recovery with specific pathways in the spinal cord (see general discussion).

Chapter 4. Anatomical observations

4.1 Introduction

The rubrospinal tract (RST) is often used in experimental models of spinal cord injury and its anatomical distribution has previously been described (Brown, 1974;Antal *et al.*, 1992). Briefly, the RST originates from the caudal 2/3 of the red nucleus and projects mainly to the contralateral spinal cord as far as lumbosacral segments. The RST terminates within Rexed's Lamina V & VI, as well as the dorsal part of lamina VII. Although the RST is mainly a contralateral tract, a small number of fibres are known to originate from the ipsilateral red nucleus. Occasionally fibres are seen terminating within the ventral horn (Kuchler *et al.*, 2002).

In humans, following injury to the spinal cord, spontaneous recovery often occurs although the mechanisms of recovery are largely unknown. In experimental models of spinal cord injury, it is often noted that changes occur in the circuitry of those pathways spared by the lesion. It has been reported that reorganisation of the RST can occur following a lesion to the corticospinal tract (CST) of the rat following treatment (Raineteau *et al.*, 2001;Raineteau & Schwab, 2001;Raineteau *et al.*, 2002).

Here, we assess the projection pattern of the RST in control animals and in those with a lesion to the corticospinal track. In this chapter, the anatomical distribution of the RST in the caudal cervical spinal cord is described. Comparisons of these projections are made with those of animals with a lesion to the corticospinal track. Axon counts of lesion and non-lesioned animals are reported and all findings are discussed.

4.2 Methods

Some control animals and some of those with a cervical spinal cord injury (approximately 11 weeks post-lesion) underwent a tract tracing procedure. Both the "spike" and injection system were calibrated for the interaural line (see Chapter 2, section 2.2 for more detailes). Animals were anaesthetised with halothane (5% for induction, maintained at 1.8-2.2%, O_2 0.8L/min). Once anaesthetised, the animals were placed on a heating blanket and the temperature was monitored with a rectal probe. The head was shaved and cleaned with a Betadine solution. The animal was placed in a stereotaxic frame (David Kopf Instruments, California) and a midline incision was made along the rat's head exposing the cranium. A burr hole was made on the right side of the skull with a dental drill (AP 2.6, LR 2.6). The dura was opened with a 27g needle and fine forceps. A 500nl Hamilton syringe attached to a needle (200μm diameter) was used to inject a 10% solution of Biotin dextran amine (BDA-10,000, Molecular Probes, Invitrogen detection technologies). The needle was positioned 2.6mm rostral to the interaural line (15º to the vertical, see Chapter 2, section 2.2). Once the needle penetrated the brain, it was advanced to a depth of 6.8mm and 200nl or 250nl of the BDA solution was injected slowly at a rate of 50nl/2mins. The needle was left in place for 10 minutes, withdrawn 100μm and left in place for a further 10mins, again withdrawn 100μm and left in place for 10mins, and finally withdrawn 100μm and left in place for 3mins. The needle was then withdrawn slowly. The skin was closed using a 3-0 vicryl suture. The animal was given 5ml of sterile saline S.C, 0.03ml Duphomux I.M, and 0.015ml vetergesic I.M. The animal was placed under a heat lamp for recovery. In all animals, recovery was monitored daily and no adverse effects were seen.

Two weeks later animals were re-anaesthetised with urethane (Sigma-Aldrich Company Ltd,1.4 g/kg, 30% in saline I.P.) and perfused intracardially with 0.9% heparinised saline followed by 4% paraformaldehyde in 0.2M phosphate buffered saline solution (PBS, pH 7.4) with 5% sucrose. The brain and spinal cord were recovered and post-fixed at 4°C in the same solution.

The spinal cord and brain were blocked. For the spinal cord, blocks (usually consisting of two segments) were produced from C1 to T2. The side ipsilateral to the injected RN was marked, as was the rostral end. The brain was placed in a brain blocker and the area of the red nucleus was identified by reference with the rat atlas (Paxinos G & Watson C, 1998) and the block was cut and extracted from the blocker. The side contralateral to the BDA injection was marked, as was the rostral end. Both the brain and spinal cord blocks were then cryoprotected with a solution of 30% sucrose in a 4% formaldehyde solution for at least three days.

Cross sections (frozen, 50μm) of the brain and spinal cord were produced using a freezing microtome (JUNG SM 1400, Leica). All sections were reacted with diaminobenzidine tetra-hydrochloride dehydrate (DAB, Sigma-Aldrich Company Ltd). For the DAB reaction, all sections were collected to phosphate buffered saline double salt (PBSD). Sections were incubated in a 50% solution of ETOH and then were washed 3 x 10mins in PBSD. The spinal cord sections underwent a blocking step in which the sections were incubated for 20mins in a solution of 30% hydrogen peroxide plus 10% methanol in 0.1M PBS. Sections were then washed 3 x 10mins in PBSD followed by a 10min wash with PBSD plus 0.3% Triton X-100 (Sigma-Aldrich Company Ltd, PBSDT). Sections were incubated overnight with ExtraAvidin-peroxidase in PBSDT (1:1000, Sigma-Aldrich Company Ltd).The following day the sections were washed 3 x 10mins with PBSD and once in phosphate buffer (PB) for

10mins. Sections were incubated for 15mins in a DAB solution intensified with 1% cobalt chloride (VWR International Ltd, UK). A solution of 30% H_2O_2 (15μl/50ml DAB) was added and the sections were carefully observed for colour change indicating the end of the reaction and the process was stopped by washing the sections with 0.1M PB. The sections were finally washed 3 x 10mins in 0.1M PB and were mounted on gelatine subbed glass slides and allowed to dry.

The brain sections were counter-stained with cresyl-violet acetate (Sigma-Aldrich Company Ltd) to allow visualisation of the injection site within the red nucleus. All slides were dehydrated stepwise through alcohol, cleared in xylene and cover-slipped with a DePex mounting medium (VWR International Ltd, UK) and allowed to dry.

Note, for the spinal cord lesioned group, two series of sections were produced for the lesion block, one was reacted with DAB and the other was stained with cresyl-violet to allow visualisation of the lesion. The remaining blocks were reacted with DAB.

The injection site and BDA traced fibres in the spinal sections were visualised using a Zeiss Axioskop microscope (Zeiss, W. Germany). All injection sites were photographed. In some cases BDA fibres from selected segments were drawn using the microscope and a drawing tube. The lesions were reconstructed in the same way. The number of fibres in the dorsolateral funiculus was counted in the control and lesioned animals. For this, three sections were chosen randomly, within half a cervical segment, for each animal and the fibres were counted at 40x.

4.3 Results

The RST is known to originate from the caudal 2/3 of the red nucleus (RN) which corresponds to the magnocellular region (Shieh *et al.*, 1983). To anatomically trace this tract, small injections of a 10% Biotin dextran amine (BDA) were made into the RN. Due to the presence of other nuclei near the RN, small injections of 200-250nl were made to minimise labelling of other brain stem nuclei. During post-mortem observations of the injection site, a hole was sometimes seen in the region of the injection site. BDA is well known for its low efficiency of staining. The RST consists of about 3000 fibres in the rat (Liu *et al.*, 1999) and here an average of 7-10% of these fibres were labelled. On average, 197 fibres of small and large diameter were counted in the DLF of the control animals (n=5) and 293 were counted in the DLF of animals with a dorsal column lesion (n=5).

Following a BDA injection to the RN, the distribution of labelled fibres was assessed in the caudal cervical spinal cord (C7). Fibres originating from the red nucleus descended into the contralateral spinal cord within the dorsolateral funiculus (DLF). Terminations of axons and collaterals were seen mainly within the intermediate grey of the contralateral spinal cord, with a few terminating within the ventral horn. A small number of fibres were seen within the ipsilateral DLF (approx 3-7 fibres). Although BDA is most commonly used as an anterograde tracer, it can also be transported retrogradely. This was evident in the current study in which some neurons were retrogradely labelled in R649 (Figure 4.1). The BDA in the injection site of this animal spread more dorsally than that of any other animal (see figure 4.3). In this animal, labelling of ascending fibres cannot be ruled out. It must be noted that retrogradely labelled cells were only seen in this animal.

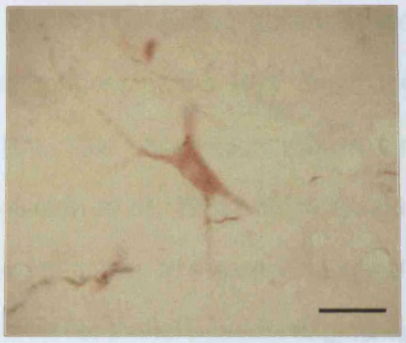


Figure 4.1 Retrogradely labelled neuron in R649.
Following a BDA injection into the RN, retrograde labelled cells were seen in the spinal cord of this animal. The cell illustrated in this figure was seen in the intermediate grey of the contralateral spinal cord. Photomicrograph taken at 40x, scale bar 25μm.

4.3.1 Projection pattern of RST in control animals

Out of seven control animals, five were chosen to be illustrated in this chapter as a result of a successful DAB reaction. Table 4.1 summarises the details of the injection site for these five animals detailing the volume injected, the distance of the injection site in the RN as calculated from the caudal edge of the RN, and the number of fibres in the DLF as counted from three random sections per animal. The caudal edge of the RN was used to calculate the distance to the injection site as the target of the injection was the RNm which is located in the caudal 2/3 of the RN.

Experiment No.	*Volume (nl)*	*Distance from caudal end of RN (μm)*	*No. of fibres in DLF*
R631	200	750	117, 141, 144 (mean 134)
R634	200	500	142, 149, 147 (mean 146)
R636	200	Approx 350	428, 394, 410 (mean 411)
R648	250	600	133, 161, 182 (mean 159)
R649	250	350	142, 139, 130 (mean 137)

Table 4.1 Summary of injection sites and fibre counts. First column indicates the animal number, second column indicates the volume of BDA injected into the red nucleus. The third column indicates the distance of the injection site from the caudal end of the red nucleus (this is rostral to the caudal end). The fourth column indicates the number of fibres in the DLF as counted from the three random sections.

Following observation of the RST projection pattern in the five animals, it became evident that the projection pattern of the RST depended on the location of the injection site within the RN. This observation was in agreement with the known somatotopy of the RN (Huisman *et al.*, 1981) which is said to be that the dorsal and medial part of the RN projects mainly to the cervical spinal cord and that the ventral and lateral part of the RN projects to more caudal segments of the spinal cord.

As shown in figures 4.2 and 4.3 and as previously described (Antal *et al.*, 1992), BDA positive fibres were seen running in the dorsolateral funiculus (DLF) contralateral to the injection site. Collaterals were seen to project densely to the intermediate grey (Rexed's laminae V, VI and VII). As in all animals, these collaterals supplied numerous boutons throughout the area of projection. Most collaterals were relatively fine but a few were remarkably thick. Few fibres were seen projecting to lamina IX (Figure 4.2B, arrow). Post-mortem observations showed that the injection sites for both of these animals were located in the dorsomedial part of the RN which corresponds to the region known to project to the cervical spinal cord. Although a similar number of fibres were labelled in these two animals (mean of 134 in R631 and 146 in R634), the RST projection in R634 (Figure 4.3) is more dense than that of R631 (Figure 4.2). The BDA injection was located in the dorsal aspect of the RN in both animals, but was located 250μm more caudally in the RN of R634. It could therefore be possible that more RNm neurons projecting to cervical spinal cord picked up the BDA in R634 resulting in denser RST labelling as compared to that of R631.

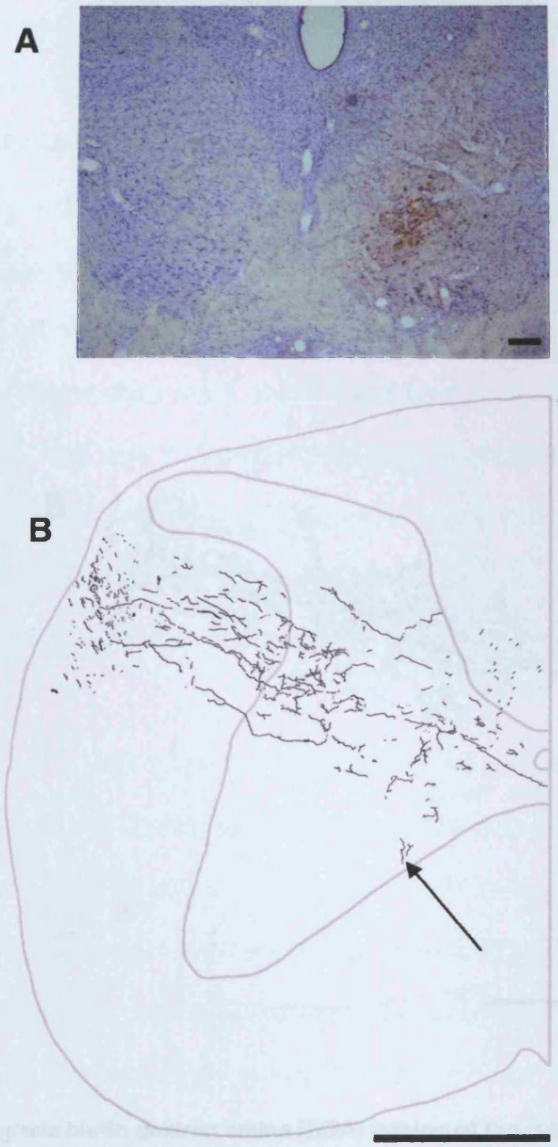


Figure 4.2 Anterograde biotin dextran amine (BDA) tracing of the rubrospinal tract (R631). **A** Photomicrograph of injection site in the red nucleus, scale bar 200μm **B** Drawing of RST fibres (C7) using a Zeiss microscope and a drawing tube, arrow shows a few fibres in lamina VIII or IX, scale bar 500μm.

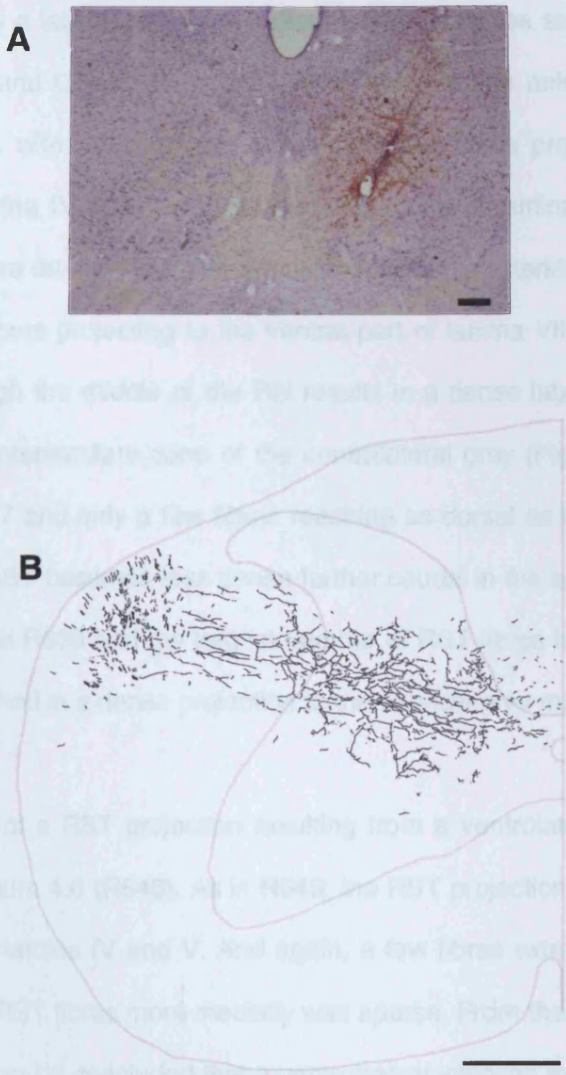


Figure 4.3 Anterograde biotin dextran amine (BDA) tracing of the rubrospinal tract (R634). **A** Photomicrograph of injection site in the red nucleus, scale bar 200μm. **B** Drawing of RST fibres (C7) using a Zeiss microscope and a drawing tube, scale bar 500μm.

Figures 4.5 & 4.5 illustrate the RST projection pattern in C5, C7, and T2 following an injection to different parts of the RN. This figure provides support for the suggestion of the somatotopy of the RN in which a ventrolateral BDA injection to the RN (Figure 4.4) is compared to that of an injection that runs through the middle of the RN (Figure 4.5). As can be seen from figure 4.4, an injection to the ventrolateral part of the RN

results in a mainly a lateral projection pattern with more fibres seen projecting to T2 compared to C5 and C7. In C5 and C7, RST fibres in this animal are seen more dorsal than usual, with a remarkable concentration of fibres projecting to the most lateral part of lamina IV and V and to the ventral part of lamina III. More caudally (T2), RST fibres are denser and their projection pattern is extended more medially in lamina VII, with fibres projecting to the ventral part of lamina VII. While an injection that passes through the middle of the RN results in a dense labelling of RST fibres within the whole intermediate zone of the contralateral grey (Figure 4.5) as can be seen in C5 and C7 and only a few fibres reaching as dorsal as lamina IV (C5). The projection of the RST becomes less dense further caudal in the spinal cord as shown at T2. Note, animal R636 had the largest number of RST fibres labelled (mean 411), which clearly resulted in a dense projection to the cervical grey matter.

Another example of a RST projection resulting from a ventrolateral injection to the RN is shown in figure 4.6 (R648). As in R649, the RST projection was most dense at the lateral part of lamina IV and V. And again, a few fibres were seen in lamina III. The projection of RST fibres more medially was sparse. From the observations of the present study, it can be concluded that a ventrolateral injection to the RN results in a more lateral projection pattern of the RST with very few fibres projecting more medially towards the central canal. The density of these fibres in the cervical spinal cord is less than those resulting from dorsal and/or medial injection to the RN.

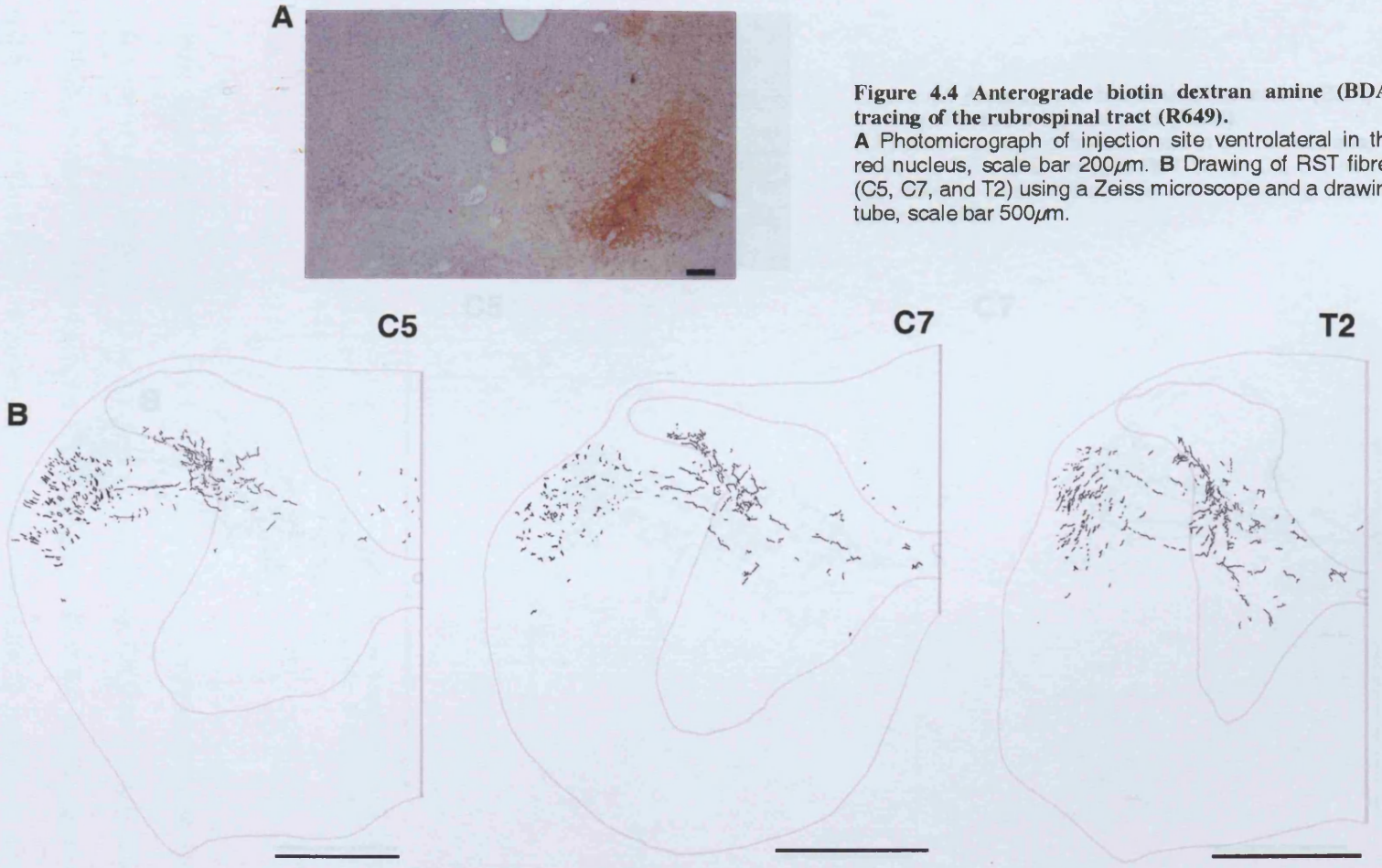


Figure 4.4 Anterograde biotin dextran amine (BDA) tracing of the rubrospinal tract (R649).
A Photomicrograph of injection site ventrolateral in the red nucleus, scale bar 200μm. **B** Drawing of RST fibres (C5, C7, and T2) using a Zeiss microscope and a drawing tube, scale bar 500μm.

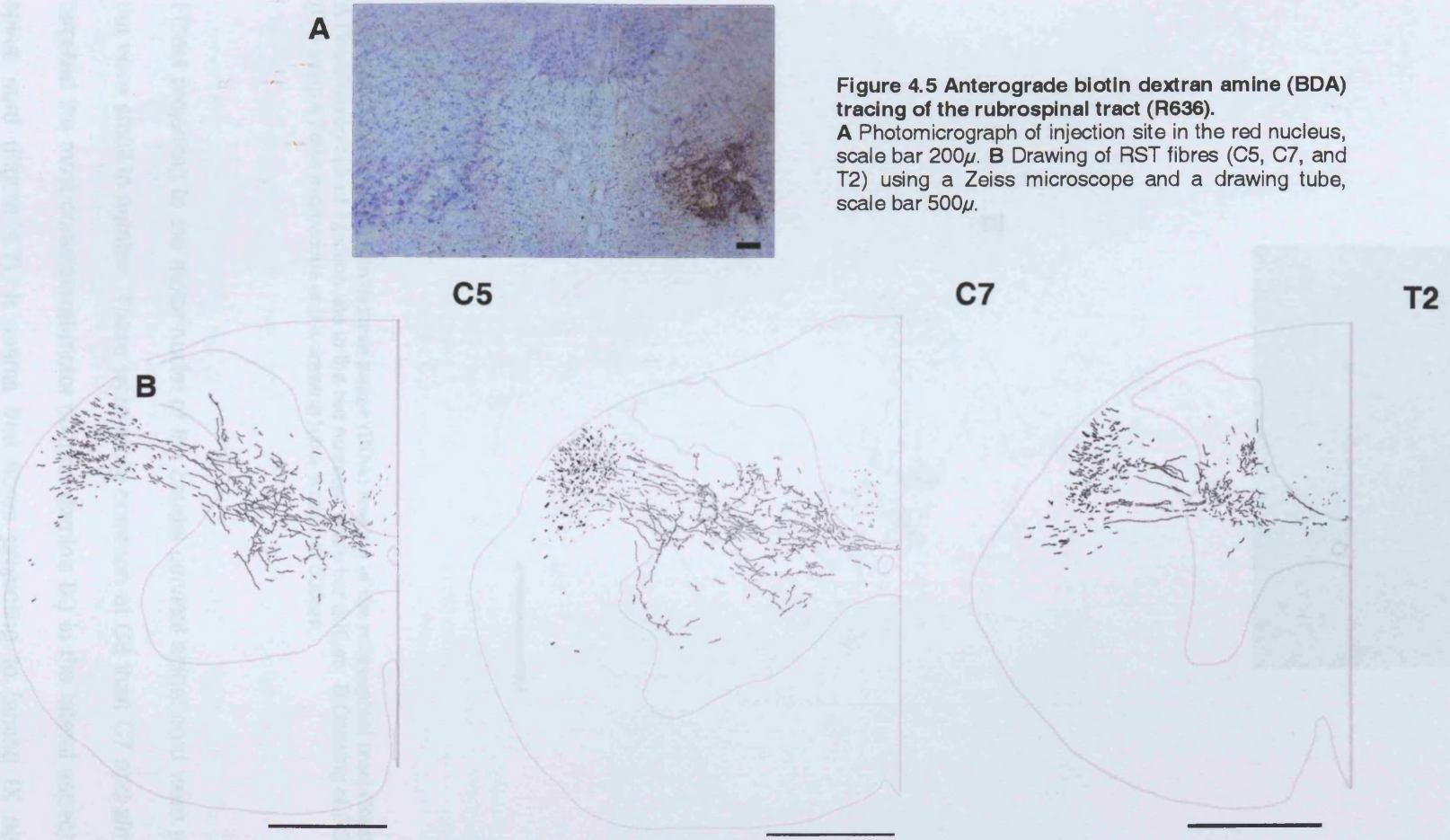


Figure 4.5 Anterograde biotin dextran amine (BDA) tracing of the rubrospinal tract (R636).
A Photomicrograph of injection site in the red nucleus, scale bar 200μ. **B** Drawing of RST fibres (C5, C7, and T2) using a Zeiss microscope and a drawing tube, scale bar 500μ.

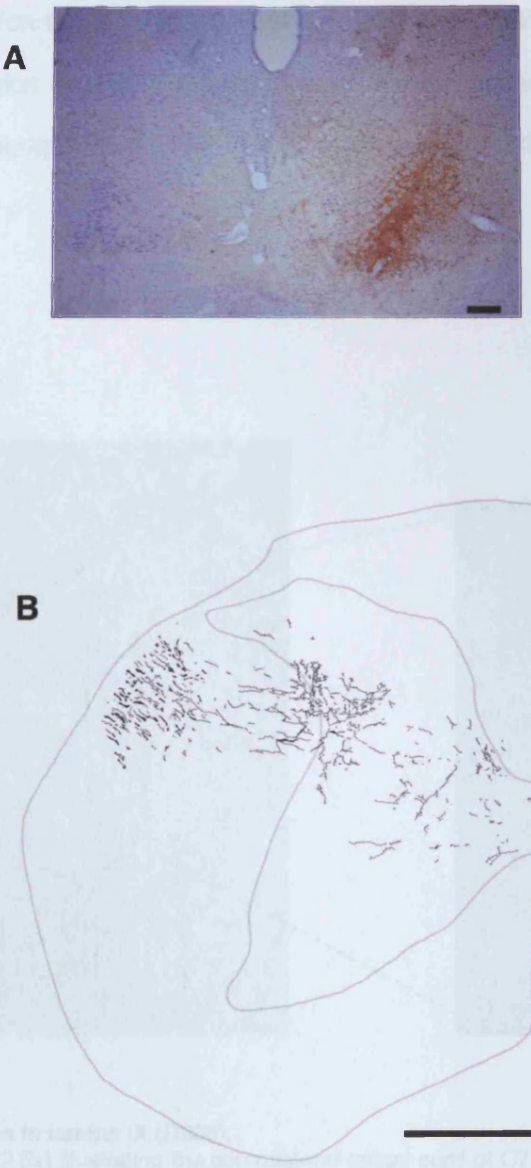


Figure 4.6 Anterograde biotin dextran amine (BDA) tracing of the rubrospinal tract (R648).
A Photomicrograph of injection site in the red nucleus, scale bar 200μm. **B** Drawing of RST fibres (C7) using a Zeiss microscope and a drawing tube, scale bar 500μm.

Fibres projecting to the motor nuclei of the caudal cervical spinal cord were present but were small in number. These were more common at C8 than C7 and almost all targeted the most dorsolateral motor column (lamina IX) in the lateral aspect of the spinal cord (Figure 4.7). It seems that fibres projecting to lamina IX occurred

following an injection to the caudal part of the red nucleus which corresponds to the magnocellular region and at a medial location within the red nucleus. Figure 4.7 shows photomicrographs taken from R636 at C7 showing RST fibres projecting to Lamina IX.

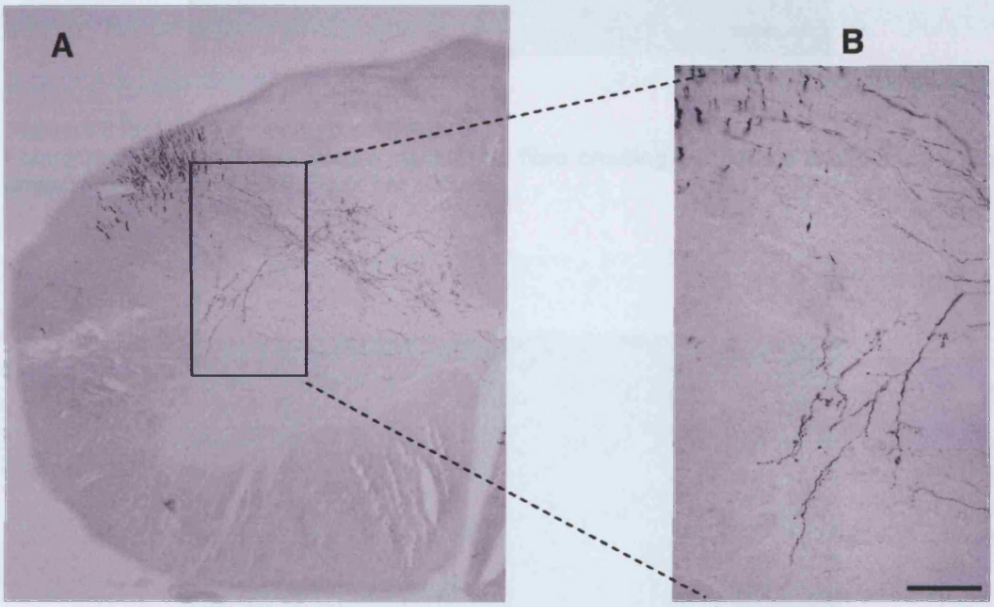


Figure 4.7 RST fibres in lamina IX (R636).
A Photomicrograph (2.5x) illustrating the contralateral spinal cord at C7 showing BDA RST fibres in the dorsolateral funiculus with collateral projecting to the intermediate zone of the spinal grey. Note RST fibres projecting to lamina IX (box). **B** Photomicrograph (20x) showing an enlarged view of RST fibres projecting to the ventral horn (lamina IX) of the contralateral spinal cord. Scale bar 100μm.

RST fibres were also seen crossing the midline, but these were very few and usually crossed the midline dorsal to the central canal (white arrow, figure 4.8). An unusual observation was that in some cases, and although rare, RST fibres were seen projecting into and crossing the dorsal columns (white arrow, figure 4.9).

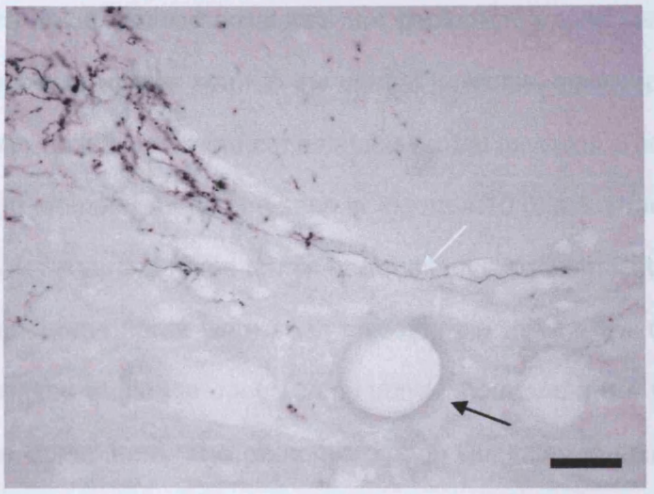


Figure 4.8 RST fibre crossing the midline.
Photomicrograph (10x) illustrating a rubrospinal fibre crossing the midline (white arrow). Black arrow indicates central canal, Scale bar 100μm.

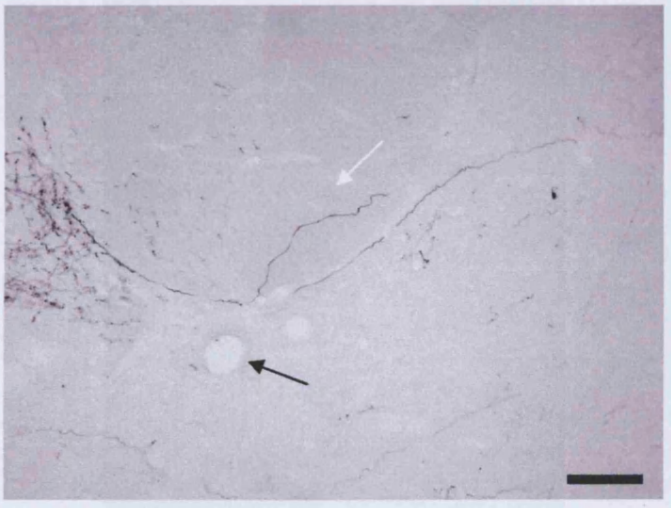


Figure 4.9 RST fibre invading the dorsal columns.
Photomicrograph (10x) illustrating BDA positive fibres projecting into the dorsal columns. Black arrow indicates central canal, white arrow indicated RST fibre in dorsal columns. Scale bar 100μm.

In addition to the observations above, labelled fibres outside the DLF were also seen. As can be seen in all previous drawings and photomicrographs, fibres were also labelled within the corticospinal part of the contralateral dorsal column. It must be emphasised here that these fibres were strictly contralateral and were always very

fine fibres which never showed collaterals (for photomicrographs see figures 4.8 & 4.9). Also, fibres were always seen in the ventral funiculus, mainly ipsilateral to the injection site, with only a few in the contralateral ventral funiculus. These fibres were always of a large diameter as can be seen in Figure 4.10 (black arrows). Collaterals from these ipsilateral ventral fibres terminated within the ipsilateral intermediate grey and ventral horn. Some fibres were seen crossing the midline (white arrow, figure 4.10) and terminating within the contralateral ventral horn and a few were also seen projecting to the dorsal horn. The photomicrograph illustrated in figure 4.10 comes from the animal with the largest number of fibres labelled in the ventral tracts. In this animal, the BDA injection was located dorsally within the RN (figure 4.3).

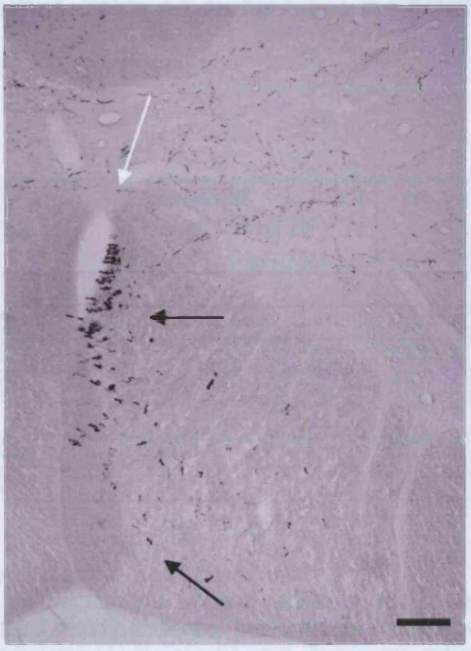


Figure 4.10 Photomicrograph (10x) illustrating BDA positive fibres in the ipsilateral spinal cord (R634).
Following an injection to the red nucleus, BDA positive fibres were seen in the ipsilateral ventromedial (black arrows) spinal cord with projections to the ipsilateral spinal cord. Some fibres were seen crossing the midline projecting to the contralateral spinal cord (white arrow). Scale bar 100μm.

4.3.2 Projection pattern of the RST in lesioned animals

Animals with a chronic lesion to the dorsal columns underwent anatomical tracing of the RST approximately 16 weeks after the lesion was made. Histological reconstructions of the lesion site demonstrated that three animals sustained a complete lesion to the dorsal columns with no damage to the ventral spinal cord and the other two sustained incomplete lesions sparing part of the dorsal columns. Animals with a lesion to the dorsal columns showed a similar termination pattern of rubrospinal fibres as compared to control animals. Although the number of animals assessed here is small, there still was no obvious evidence of sprouting following a lesion to the dorsal columns. Table 4.2 summarises the details of the BDA injections and the number of fibres seen in the DLF.

Experiment No.	*Volume (nl)*	*Distance from caudal end of RN (μm)*	*No. of fibres in DLF*
Complete lesions			
R606	200	400	469, 421, 453 (mean 448)
R646	200	250	115, 123, 131 (mean 123)
R647	200	250	197, 184, 223 (mean 201)
Incomplete lesions			
R607	200	300	363, 332, 348 (mean 348)
R608	200	400	351, 370, 319 (mean 347)

Table 4.2 Summary of injection sites and fibre counts.
First column indicates the animal number, second column indicates the volume of BDA injected into the red nucleus. The third column indicates the distance of the injection site from the caudal end of the red nucleus (this is rostral to the caudal end). The fourth column indicates the number of fibres in the DLF as counted from the three random sections.

As in control animals, the projection pattern of the RST was restricted mainly to lamina V, VI and VII with very few fibres projecting more dorsally to lamina III and IV or ventrally to lamina IX.

The following figures illustrate the injection site, lesion reconstruction (C5), and drawings of the RST projection for all lesioned animals. As in control animals, the number of fibres projecting to the motor nuclei was few. A drawing of the RST projection at C7 is shown in figure 4.11C with an enlarged view of the fibres seen in lamina IX shown in figure 4.11D. It must be noted here that although this is the heaviest projection observed in the lesioned group, this animal also had the largest number of fibres (mean 448) and the injection site was located medially within the RN. This animal is comparable with R636 from the control group, in which both animals had a similar BDA injection site, resulted in the largest number of fibres labelled in the DLF and the densest RST projection pattern, and also showed the most projections to the lamina IX (cf. R636 in table 4.1, and figures 4.5 & 4.7).

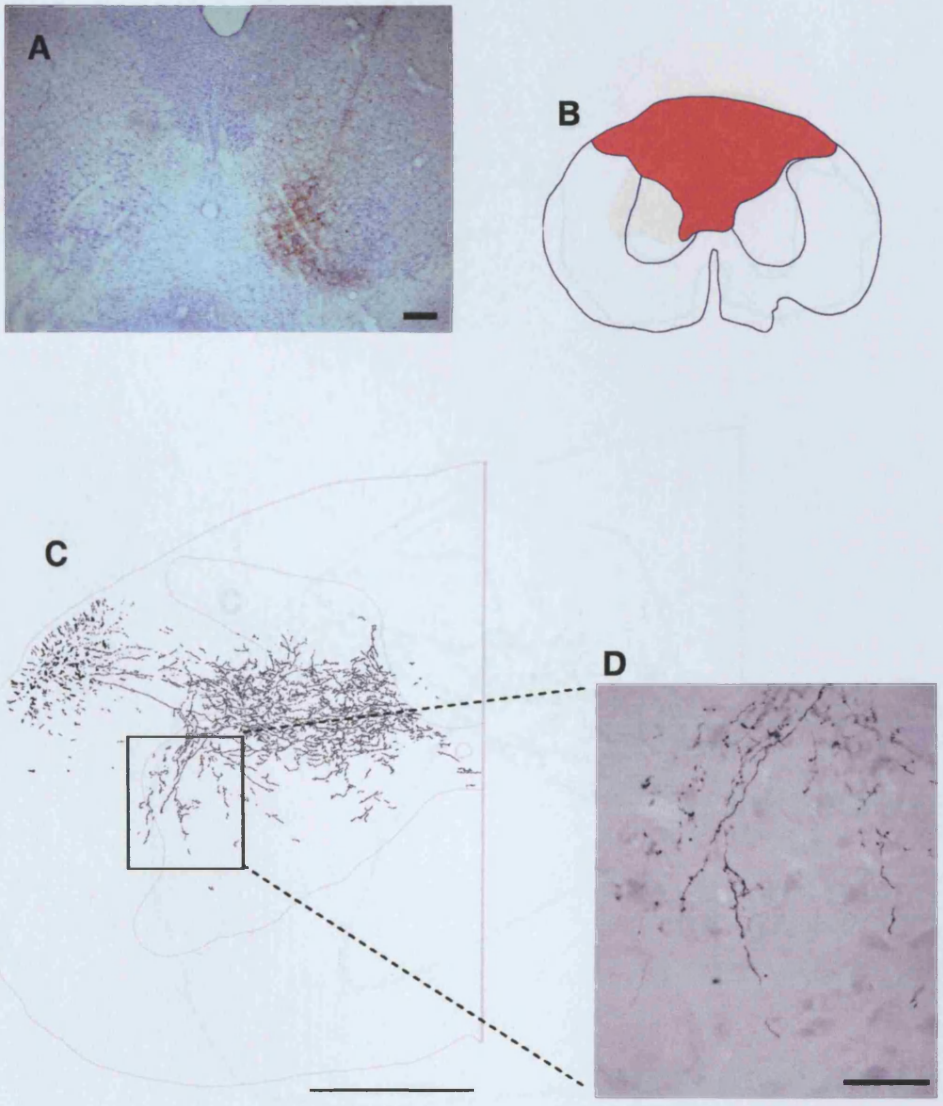


Figure 4.11 Anterograde biotin dextran amine (BDA) tracing of the rubrospinal tract (R606). **A** Photomicrograph of injection site in the red nucleus, scale bar 200μm. **B** Reconstruction of dorsal column lesion at C5. **C** Drawing of RST fibres (C7) using a Zeiss microscope and a drawing tube, scale bar 500μm. **D** Photomicrograph of BDA traced RST fibres projecting to the ventral horn (lamina IX), 20x, scale bar 100μm.

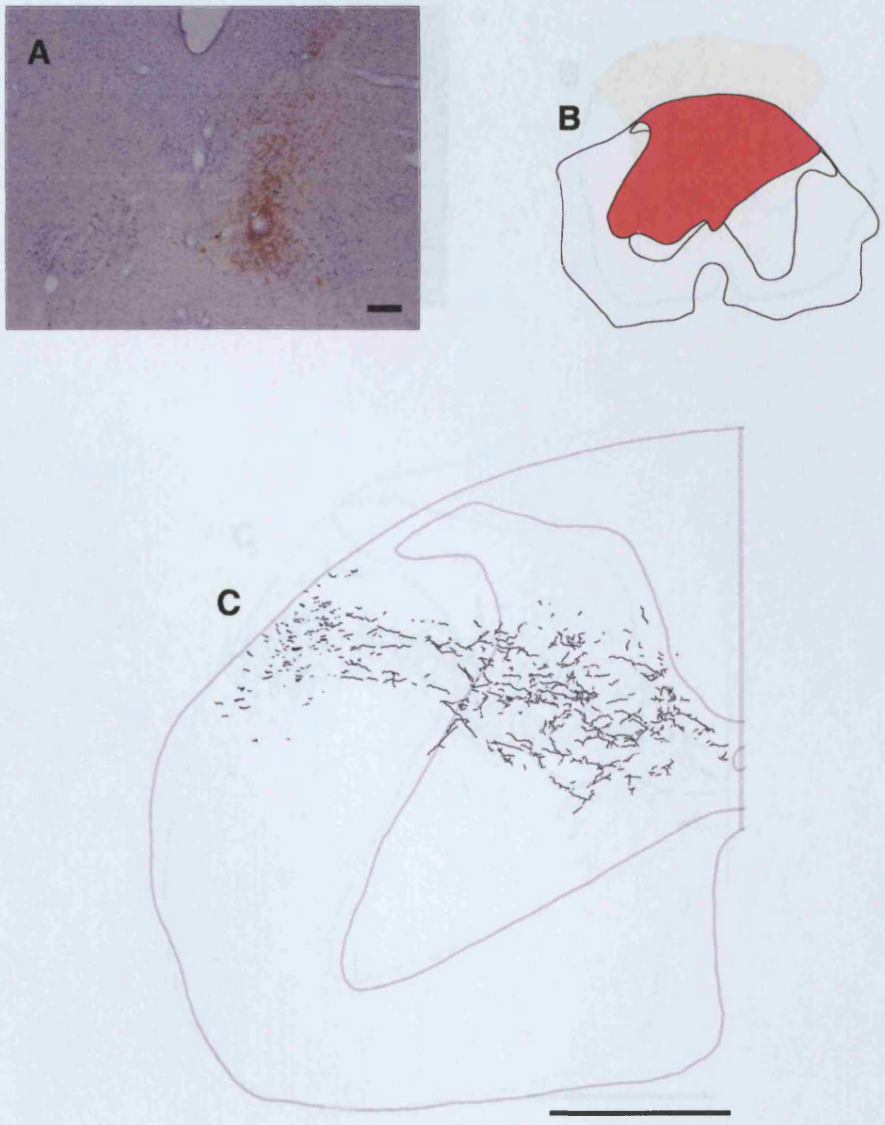


Figure 4.12 Anterograde biotin dextran amine (BDA) tracing of the rubrospinal tract (R646). **A** Photomicrograph of injection site in the red nucleus, scale bar 200μm. **B** Reconstruction of dorsal column lesion at C5. **C** Drawing of RST fibres (C7) using a Zeiss microscope and a drawing tube, scale bar 500μm.

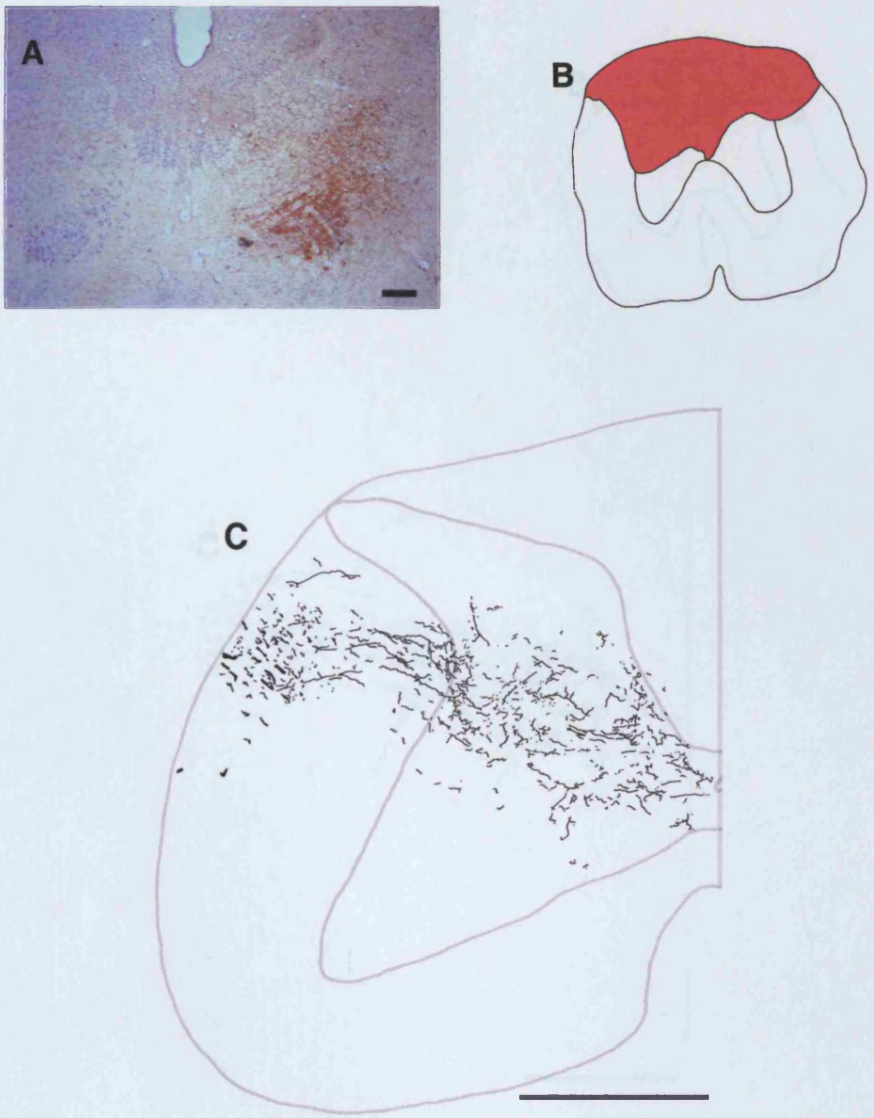


Figure 4.13 Anterograde biotin dextran amine (BDA) tracing of the rubrospinal tract (R647). **A** Photomicrograph of injection site in the red nucleus, scale bar 200μm. **B** Reconstruction of dorsal column lesion at C5. **C** Drawing of RST fibres (C7) using a Zeiss microscope and a drawing tube, scale bar 500μm.

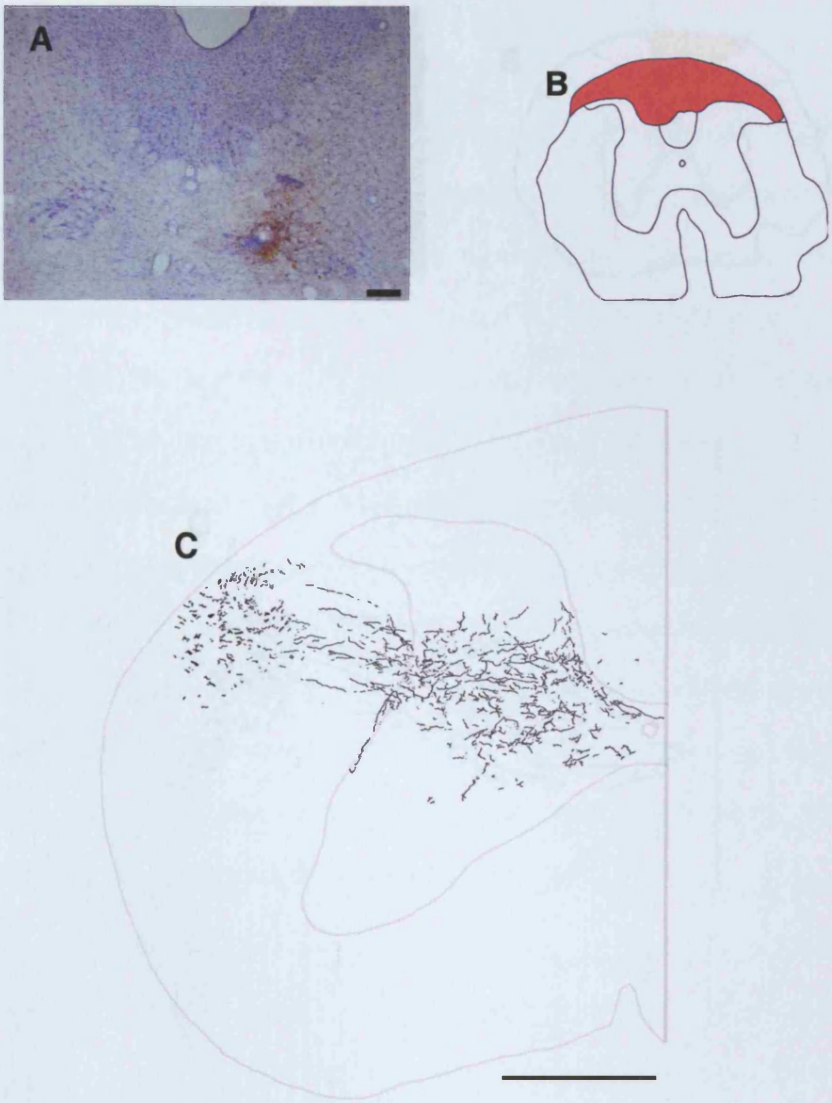


Figure 4.14 Anterograde biotin dextran amine (BDA) tracing of the rubrospinal tract (R607). **A** Photomicrograph of injection site in the red nucleus, scale bar 200μm. **B** Reconstruction of dorsal column lesion at C5. **C** Drawing of RST fibres (C7) using a Zeiss microscope and a drawing tube, scale bar 500μm.

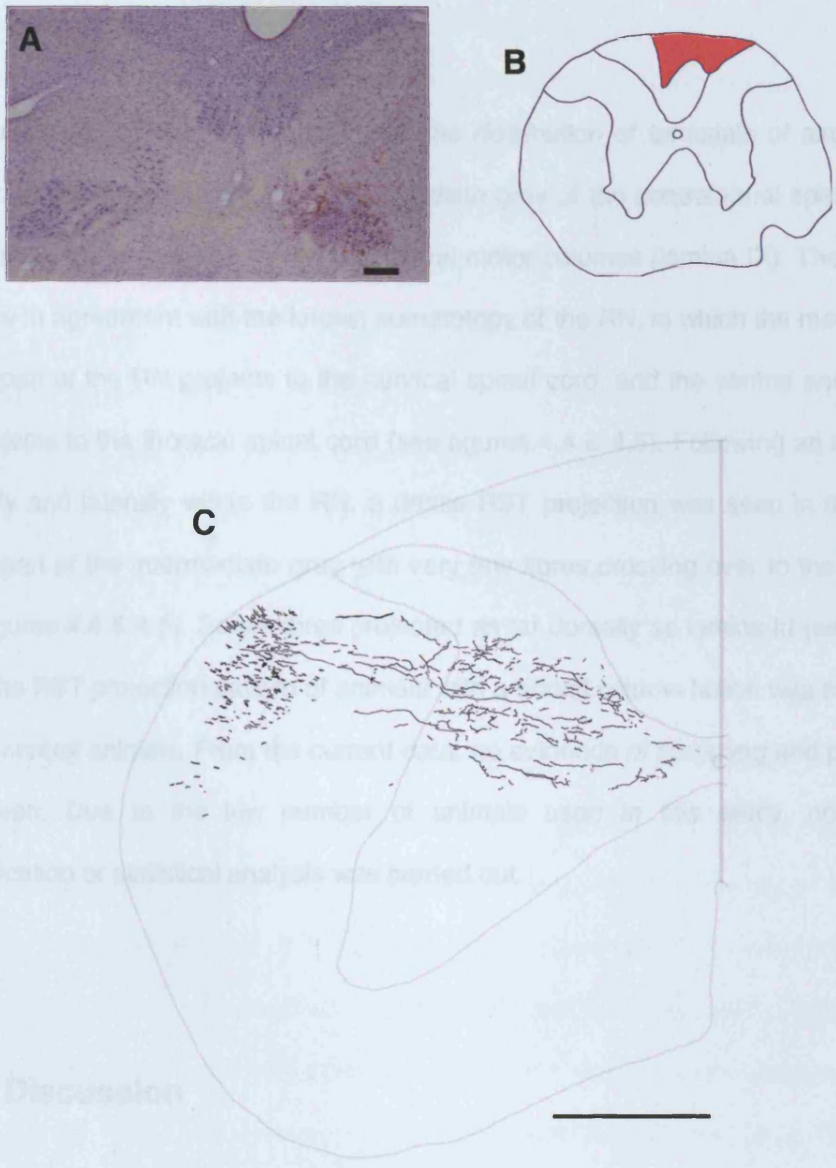


Figure 4.15 Anterograde biotin dextran amine (BDA) tracing of the rubrospinal tract (R608). **A** Photomicrograph of injection site in the red nucleus, scale bar 200μm. Photomicrograph is taken 150μm caudal to the centre of the injection due to a large hole which obscured the vision of the injection track. **B** Reconstruction of dorsal column lesion at C5. **C** Drawing of RST fibres (C7) using a Zeiss microscope and a drawing tube, scale bar 500μm.

4.3.3 Summary

Following a tracer injection into the RN, the distribution of terminals of axons and collaterals was seen mainly in the intermediate grey of the contralateral spinal cord. Few fibres projected to the most dorsolateral motor columns (lamina IX). The results here are in agreement with the known somatotopy of the RN, in which the medial and dorsal part of the RN projects to the cervical spinal cord, and the ventral and lateral RN projects to the thoracic spinal cord (see figures 4.4 & 4.5). Following an injection ventrally and laterally within the RN, a dense RST projection was seen in the most lateral part of the intermediate grey with very few fibres crossing over to the midline (see figures 4.4 & 4.5). Some fibres projected as far dorsally as lamina III (see figure 4.4). The RST projection pattern of animals with a dorsal column lesion was similar to that of control animals. From the current data, no evidence of sprouting and plasticity was seen. Due to the low number of animals used in this study, no formal quantification or statistical analysis was carried out

4.4 Discussion

4.4.1 Summary of results

Following an injection of Biotin dextran amine (BDA) into the red nucleus (RN), labelled rubrospinal fibres were seen mainly within the contralateral dorsolateral funiculus (DLF) with only a few fibres seen in the ipsilateral DLF. Terminals of rubrospinal axons and collaterals were seen mainly within the intermediate grey of the contralateral spinal cord. Very few terminals were seen dorsal to lamina IV and ventral to lamina VII. A few fibres were also seen crossing the midline. Fibres projecting to motor nuclei were seen in C7 and C8 but were more common in C8.

These fibres usually targeted the most dorsolateral motor column (lamina IX). In both the lesioned and control group of animals, a single animal out of each group stood out with the largest number of fibres projecting to lamina IX (R636 & R606). The locations of the injection sites in these two animals were similar (350μm and 400μm rostral from the caudal end of the RN).

BDA labelled fibres were also seen outside the DLF. These were in the dorsal (more common) and medial part of the ventral funiculus mainly ipsilaterally with only a few contralaterally. These fibres were higher in number if the BDA injection was located dorsally in the RN. The possible origin of these ipsilateral fibres will be discussed below.

Following a bilateral lesion to the dorsal columns, the distribution pattern of the RST was similar to that of the control animals. No quantification was carried out due to the low number of animals with a complete lesion and anatomical tracing (n=3). Although in theory, 5 lesioned animals (3 complete, 2 incomplete) could be compared against the control animals (n=5). However, the injection sites in the lesioned animals were medially within the RN, and only three control animals had an injection site within the medial aspect of the RN. Therefore if a comparison were to be made, the three control animals with medial injection sites would have to be compared against the five lesioned animals, in which only three had a complete lesion. It must also be noted that although the number of fibres in the DLF were often similar (see table 4.1 & 4.2 in results), the density of projection varied. From the observation of the RST projections and from the drawings it can be hypothesised that a significant difference would not be seen, and on this basis no formal quantification was made. However, these data can be used in future anatomical tracing studies of normal and lesioned animals.

4.4.2 Somatotopy in the projection pattern of the RST

The distribution of the RST projection was in agreement with the known somatotopy of the red nucleus in which the medial and dorsal part of the RN projects to the cervical spinal cord and the lateral and ventral part of the RN projects to more caudal segments (Huisman *et al.*, 1981). In the current study, it was clearly evident that if the BDA injection was dorsal and/or medial in the RN, the projection pattern of the RST was denser in the cervical spinal cord as compared to the thoracic cord (T2). On the other hand, if the BDA injection was ventral and/or lateral in the RN, the projection pattern of the RST was denser at the thoracic level. It was observed that following a lateral and/or ventral injection, the resulting projection pattern of the RST was that of a more lateral and dorsal one. With an injection at this particular location in the RN, a remarkable concentration of RST fibres was seen at the most lateral part of lamina IV and V with some fibres projecting as far dorsal as lamina III. This lateral projection has not been previously described. Therefore somatotopy within the RN may not only contribute to the rostral vs caudal projection pattern within the spinal cord but also in the medial vs lateral projection pattern.

4.4.3 Hypothesis for non RST labelling

Following an injection of BDA into the red nucleus, rubrospinal fibres were labelled as previously described (Antal *et al.*, 1992). However, fibres were also labelled that were not of the rubrospinal tract. This could be due to the spread of the BDA resulting in the labelling of other brain stem nuclei (although every care was taken to minimise this), or of fibres of passage, damaged axons or the BDA could have been picked up by terminals and transported anterogradely or retrogradely. Evidence exists for BDA uptake by terminals and by damaged axons caused by the injection, but it is still not

clear whether uptake occurs by fibres of passage (Reiner *et al.*, 2000). BDA is known to be transported not only anterogradely as in this study, but also retrogradely (Chen & Aston-Jones, 1998;Reiner *et al.*, 2000). The transport of BDA either retrogradely or anterogradely depends on its molecular weight and the pH of the delivery vehicle. Low molecular weight BDA (3K) delivered in an acidic vehicle is preferentially transported retrogradely, while high molecular weight BDA (10K) seems to be preferentially transported anterogradely, but it must be noted that it still is not exclusively an anterograde tracer. In this study, 10K BDA was used and it is therefore expected that the BDA would be transported mostly anterogradely. However, in one particular animal, retrorogradely labelled cells were seen in the spinal cord (Figure 4.1). The fact that BDA can be transported either retrogradely or anterogradely is a major disadvantage of using BDA as an anterograde tracer. This causes a problem in that anterograde labelling and labelling of collaterals of retrogradely labelled neurons may not be easily distinguished. However, this is not such an issue in this thesis, as retrogradely labelled neurons were seen in only one animal.

Non-RST fibres were seen mainly in the ventral funiculus of the ipsilateral spinal cord. Some fibres were also seen in the contralateral ventral funiculus, but these were few. The non-RST fibres projected bilaterally into the ventral horn and intermediate grey with an ipsilateral dominance. It is known that fibres from the ventral uncrossed CST are seen within the medial aspect of the ventral funiculus (Brosamle & Schwab, 1997). However, it also known that CST fibres are of small diameter (Brosamle & Schwab, 2000) and as the fibres seen in the ventral funiculus of this study are of a large diameter, the CST is immediately excluded as a possible identity for these ventral fibres. However, they could have originated from a number of brainstem nuclei such as:

1. The Ipsilateral mesencephalic reticular formation
2. The interstitial nucleus of Cajal (INC)
3. Field H of forel (FR)
4. The periaqueductal grey (PAG)

A study aimed at localising spinal cord projecting neurons in adult rats showed that following an injection of horseradish peroxidase into the cervical spinal cord, neurons were labelled in the areas adjacent to the red nucleus, the PAG, and accessory oculomotor nuclei which include the INC and the mesencephalic reticular formation located adjacent to the PAG. All neurons were labelled bilaterally with an ipsilateral dominance (Leong *et al.*, 1984).

It has also been reported that fibres from the INC and adjacent reticular formation are seen in the dorsal part of the ventral funiculus and project bilaterally to the medial parts of the upper cervical ventral horn. Further caudally, labelled fibres are seen in the medial part of the ventral horn. Neurons from field H of forel (FR) are also located in the areas adjacent to the RN and project via the ventral part of the ventral funiculus to the lateral part of the upper cervical ventral horn. At lower cervical levels, fibres are distributed to the medial part of the ventral horn and projections from the FR to the thoracic and more caudal segments of the spinal cord are sparse (Holstege & Cowie, 1989;Holstege, 1991)

In the cat, neurons from the lateral PAG at levels caudal to the red nucleus project to the spinal cord via the central tegmental tract into the ipsilateral ventral and ventrolateral funiculi of the spinal cord and are sparse beyond T3 (Holstege, 1991). A few descending fibres from the PAG are also seen in the DLF. At C1 fibres terminate in the lateral part of the intermediate zone and at C2-C4 fibres terminate more medially in the intermediate zone. Further caudally, fibres terminate in the medial part

of the ventral horn. Some fibres terminate in lamina X and the upper thoracic intermediolateral cell column. These terminations are probably derived from those fibres that descend in the DLF.

From the reported findings of other groups and from the anatomical observation of the location of non-RST fibres in the ventral funiculus, it can be therefore hypothesised that fibres seen in the dorsal part of the ventral funiculus are probably those of the INC and adjacent reticular formation, while those seen in the more lateral and ventral part are those of the FR (less common).

As for those fibres seen in the contralateral dorsal columns of the present study, it is known that axons of the CST arise from the large layer pyramidal cells (layer V) of the sensorimotor cortex and project to the ventral aspect of the brain stem while giving off collaterals to many nuclei including the red nucleus. Once The CST axons pass the olivary complex, they decussate and project to the spinal cord. The mammalian red nucleus receives input from the ipsilateral sensorimotor cortex. In cats, it has been reported that 80% of the motor cortical projections to the RN come from collaterals of pyramidal tract neurons (Futami *et al.*, 1986). Following an injection of horseradish peroxidase conjugate with wheat germ agglutinin (HRP-WGA) into the sensorimotor cortex of rats, synaptic terminals were labelled in the ipsilateral red nucleus which were located on small diameter dendrites of the parvocellular region of the red nucleus (Naus *et al.*, 1985). It is therefore possible that these axons that originate from the sensorimotor cortex have taken up BDA injected into the red nucleus which would lead to the presence of CST fibres in the dorsal columns.

4.4.4 Ideal location for RN injection and assessment of RST projection pattern

The location of the BDA injection into the RN varied between animals. It became apparent that injecting BDA at a consistent site between animals was not simple. Injection of BDA into the dorsal aspect of the RN resulted in the largest number of ipsilateral non-RST fibres labelled. An injection slightly medial to the RN resulted in the most specific labelling of the RST. In two animals the projection pattern of the RST in the caudal cervical spinal cord appeared to be a more lateral one, where the fibres were more densely labelled at the lateral edge of the intermediate zone with not many fibres labelled more medially towards the central canal. It became evident that this distribution of the RST was in agreement with the known somatotopy of the red nucleus. And therefore, to target the cervical spinal cord with the most accuracy, an injection to the caudal part of the RN (magnocellular) at a more medial location would probably be ideal.

Investigators carrying out anatomical tracing of the RST must take care when assessing the projection pattern as to not mistake those fibres crossing the midline from the ipsilateral ventral fibres for rubrospinal fibres. However, it does seem rather strange that published findings from other investigators of anatomical tracing of the RST do not report labelling of non-RST fibres. The injection sites in the current study were small and precise with no extensive spread of BDA, but fibres were still always seen in the contralateral dorsal columns and the ventral funiculus of the spinal cord (mainly ipsilaterally). To the best of my knowledge, only one paper reports corticospinal labelling following a BDA injection to the RN (Fabes *et al.*, 2006). To deal with this, they used a viral vector which only infected midbrain neurons in place of the BDA to trace the RST. However, in a recent study of rubrospinal projections, a photomicrograph of the spinal cord at C2 and C6 showing the projection pattern of

the RST following an iontophoretic BDA injection into the dorsal part of the RN showed labelled fibres in the contralateral dorsal columns (Yasui *et al.*, 2001). However, there was no reference to these fibres in the text.

A rather important question to ask here is why other investigators do not report the labelling of dorsal column and/or ventral fibres. When sprouting or plasticity is reported by other investigators, do they take into consideration fibres projecting from the ventral funiculus? If they do not show or talk about these fibres, can one be confident about their anatomical descriptions. In this study, only a small volume of BDA was injected into the RN which resulted in a precise injection site. But fibres other than that of the RST were still seen (for example, compared to 2μl injected bilaterally into the RN by Koda *et al* 2004, 1μl unilaterally by Liu *et al* 1999, 0.5μl by Tobias *et al* 2003, 1μl by Xiao *et al* 2005, and 0.3μl by Xiao *et al* 2007). Most investigators do not show the injection site in the RN and do not report the labelling of non-RST fibres. An iontophoretic injection may results in less spread of BDA and a more specific injection as shown by Kuchler *et al.* However, as shown in Yasui *et al* (but not actually reported by the authors), CST fibres were still labelled after such an injection. The findings of this study strongly suggest that labelling of the RST, with no labelling of other tracts is difficult to achieve. When investigators report plasticity, for example sprouting to the ventral horn, or an increase in RST fibres crossing the midline, one must be critical of these findings and must investigate the evidence provided for such a result, as it is clearly shown here that the labelled ventral fibres project to the grey matter and some do indeed cross the midline. This is even more imperative when photomicrographs of the injection site or high magnification photomicrographs of the whole spinal cord (i.e. contralateral and ipsilateral) are not shown.

It was reported in the results section of this chapter that no evidence of sprouting or plasticity was seen in the animals with a lesion to the dorsal columns. Even though a small number of animals were used in this study, it is obvious that there is no evidence of extensive sprouting from the rubrospinal fibres. Taking this into account, and the issue of varied injection sites and the varied number of fibres in the DLF even with a similar injection site, no formal quantification was carried out.

Although sprouting is known to occur following an injury to the spinal cord, in the RST, this is reported to occur following therapeutic intervention. It was reported that following a bilateral lesion to the CST at the level of the medulla oblongata, the number of RST fibres projecting to the ventral horn increased significantly in animals treated with mAb-IN1, but did not in untreated animals (Raineteau *et al.*, 2002). Hendriks and colleagues investigated CST sprouting following a bilateral lesion at T8 to the CST or the RST, or following a hemisection destroying both the CST and RST. It was reported that spontaneous CST sprouting occurred rostral to the lesion only after the CST lesion (Hendriks *et al.*, 2006). In another study, a bilateral lesion to the dorsal columns was carried out at C3 and sprouting was assessed four weeks post lesion. Following the lesion, significant sprouting occurred from the ventral uncrossed CST onto the medial motor neuron pool (Weidner *et al.*, 2001). Bareyre and colleagues also showed that CST fibres can sprout following a mid-thoracic dorsal hemisection (Bareyre *et al.*, 2004). They reported that at three weeks post injury, there was a four-fold increase in CST collaterals in the grey matter but had decreased at twelve weeks post injury. At both three weeks and twelve the number of collaterals was significantly more compared to controls. The CST collaterals were reported to contact propriospinal interneurons rostral to the dorsal hemisection thereby connecting the injured axons to the caudal spinal cord. In a more recent study, sprouting in the CST was assessed following a mid thoracic dorsal over-

hemisection in which only the left ventral quadrant was spared (Vavrek *et al.*, 2006). In their study, no sprouting of injured CST fibres occurred or re-routing via propriospinal interneurons as reported by Bareyre *et al.* They applied NT3 at the cervical enlargement and did not see an increase in sprouting. When BDNF was applied to the cell bodies of lesioned CST neurons, a significant increase in sprouting and in the number of contacts with propriospinal neurons was seen.

It could therefore be the case that injured fibres are more likely to sprout than non-injured ones. In the case of the reported sprouting from the un-injured ventral CST, it could be possible that sprouting was more profound due to the low number of fibres in the ventral CST, in which the occurrence of sprouting is easy to identify. It seems that for plasticity to occur within the RST, therapeutic interventions are necessary, such as the administration of mAb-IN-1 or neurotrophins.

4.4.6 Conclusion

The present study investigated the anatomical projection pattern of the rubrospinal tract in control animals and in those following a cervical spinal cord injury. In control animals, RST fibres were seen mainly in the intermediate zone of the contralateral spinal cord. If the injection within the red nucleus was at a more lateral and/or ventral location, rubrospinal fibres reached as far dorsal as lamina III. A few fibres were seen projecting to the most dorsolateral motor columns (lamina IX), which were more common at C8. Non RST fibres were always seen in the ventral funiculus of the spinal cord and were denser following an injection dorsally within the RN. These probably represent fibres that originate from the interstitial nucleus of Cajal and adjacent reticular formation and/or Fields of Forel. Following an injection to the RN, fibres were also labelled in the CST part of the dorsal columns. Although a small

group of animals were used to assess plasticity following a lesion to the cervical spinal cord, there was no evidence of sprouting in this group. The data presented in this chapter can be used in further studies assessing plasticity in the RST in animals following a lesion to the dorsal columns and following therapeutic interventions.

Chapter 5. General Discussion, Conclusions, and Future studies.

5.1 Background

Spinal cord injury (SCI) results in the loss of sensation and voluntary movements below the level of the lesion. The extent of this loss depends on the size of the lesion and the level of the injury. Larger lesions can results in permanent disabilities whereas spontaneous functional recovery can occur if the lesions are smaller. High spinal lesion result in tetraplegia whereas lower spinal lesions results in paraplegia. Following SCI, spontaneous functional recovery can occur but it is often unknown if this recovery is due to regeneration of damaged fibres or reorganisation of spared pathways. If regeneration or reorganisation of pathways was to occur, a question which often remains to be answered is: do the fibres make functional connections? The present study combined electrophysiological, behavioural, and anatomical techniques to investigate changes in one pathway following a lesion to another. Electrophysiologically, and anatomically, the projection pattern of the RST in the caudal cervical spinal cord was investigated. Following a lesion to the corticospinal tract, the changes in the synaptic actions and the anatomical projection pattern of the RST were assessed. And finally, the behavioural consequences of destroying the CST were examined.

The rubrospinal tract (RST) is known to originate from the caudal 2/3 of the red nucleus. It decussates at the level of the tegmentum and runs in the DLF reaching lumbosacral segments. Termination of RST fibres are usually seen within the intermediate grey of the spinal cord with very few collaterals projecting to the ventral horn. The corticospinal tract (CST) originates from the large Betz neurons in layer V

of the sensorimotor cortex. The CST decussates at the level of the medulla and its fibres descend along the spinal cord reaching lumbosacral segments. The main part of the CST runs in the ventral part of the dorsal columns. It has been proposed that the RST can compensate for damage to the CST following an injury to the spinal cord.

5.2 Summary of results

The current study provided valuable information on the electrophysiological and anatomical projection pattern of the RST, specifically onto caudal segments of the cervical spinal cord. The physiological descriptions of the synaptic actions of the RST have yet to be described in the rat. Anatomically, the RST projection pattern has previously been described (Brown, 1974;Antal *et al.*, 1992) but this thesis provides a more detailed description, and some important observations are reported. Functional assessments of recovery following a cervical spinal cord injury are also reported.

The electrophysiological studies of the projection pattern of the RST in caudal cervical spinal cord demonstrated that synaptic actions of the RST are usually seen in the intermediate grey of the contralateral spinal cord. However, if the stimulation site is in the caudal and/or ventral part of the RN, or caudal to the RN, synaptic actions are seen in the ipsilateral spinal cord. This most likely corresponds to the activation of RST fibres from the RN contralateral to the simulation site. This was more apparent in the lesioned animals in which the stimulation site of four animals was caudal to the RN. It became evident that anatomical descriptions of the lesions did not reveal the full extent of lesion severity. Anatomically, some animals showed a lesion to the dorsal columns with no extension of lesion pathology to the DLF. However, some CDP records were dominated by positivity which indicated

physiological damage to the RST. In others, where anatomical descriptions of lesion extent extended beyond the dorsal columns and into the DLF, the records of the CDP were normal. However, in all animals, with signs of anatomical or physiological damage to the RST, synaptic potentials were still recorded which demonstrates that although damaged, the RST was still physiologically functional. This observation must be taken into account when assessing functional recovery and attributing the recovery to specific pathways.

Following anatomical tracing of the RST, it was demonstrated that the distribution of axon terminals and collaterals was restricted mainly to the intermediate grey of the contralateral spinal cord with few fibres projecting dorsally beyond lamina IV and ventrally beyond lamina VII. In general, the anatomical projection pattern of the RST corresponded to that determined physiologically. The anatomical projection pattern supported the suggestion of somatotopy in the RN, with an additional observation here of a more lateral projection following tracer injections ventral and/or lateral within the RN. A corresponding correlation was not seen in the physiological experiments, and may be explained by the spread of the stimulus resulting in activation of RN neurons around the stimulation site, either synaptically via interpositorubral fibres or directly.

It was also noted that following a BDA injection into the RN, fibres were seen in the CST part of the dorsal columns and in the ventral funiculus of the spinal cord. The ventral fibres were higher in number when the BDA injection was dorsal within the RN. These fibres probably represent those of the reticulospinal system, which would also explain the observation of ventral synaptic potentials in one animal (R478) in which post-mortem examination of the stimulation site demonstrated to have been dorsal to the red nucleus.

BDA fibres projecting to lamina IX were more common at C8 than C7. Only one intraspinal map was made at C8 in which the focus of negativity spread towards the most dorsolateral lamina IX.

Spontaneous functional recovery was assessed using three behavioural tests, the pellet retrieval test, the cylinder test, and the sticker removal test. Out of these three tests, the pellet retrieval test proved to be the most discriminatory. Assessments of the success rate of pellet retrieval test demonstrated that animals with a lesion to the dorsal columns recovered to pre-lesion levels. However, it was noted that if the lesion extent spread to the dorsolateral funiculus and/or the ventral spinal cord, animals did not recover to pre-lesion levels.

Following a lesion to the dorsal columns, destroying the main component of the corticospinal tract, the distribution of the synaptic actions and the anatomical projection pattern of the RST were similar to that seen in the control animals with no evidence for plasticity in the RST.

5.3 Critical evaluation of the electrophysiological experiments

During the electrophysiological experiments, there were a number of sources of variability either between or within experiments. One issue can be excluded, which is the reproducibility of the field potential recordings. Although no single isopotential map was sampled twice within one experiment, during some experiments, the recording electrode in the spinal cord was removed and relocated to a number of tracks recorded earlier in the

experiment, and the field potentials observed were the same as recorded previously.

During the experiments, the intensity of the stimulus in the RN was chosen in order to produce a comparable CDP in the spinal cord. Although different stimulus intensities were used, it was more important here to assess the spatial distribution of synaptic terminals. Also, varying the stimulus intensity allowed us to separate the early volley from the synaptic one. However, the variation in stimulus location is a serious criticism here due to the small number of animals reported in this study. On the other hand, the variation also allowed us to examine the different modes of activation, i.e. synaptic and direct and allowed us to report the best stimulation site for specific activation of the RN. Establishing these criteria are crucial, as this has only been reported in the cat to date. Although there is no direct evidence here as to which fibres mediate the synaptic volley, the accumulative evidence for the interpretation presented above is strong and the argument provided here is similar to that put forward with respect to the cat.

As in any electrophysiology experiments, the level of anaesthesia will have an effect on the recorded field potentials, especially the post synaptic field potentials. The anaesthetic level was monitored throughout the experiment and a serious effort was made at keeping it at a constant level.

It will also have been perfectly clear from the electrophysiological results that some difficulties were incurred in fitting the isopotential maps onto the

standard section from the rat atlas. Severe shrinkage occurred in some of the histological sections. It was also often the case that the dorso-ventral aspect of the maps did not fit well as the histological section was flattened. For these reasons, the most accurate compromise was to choose corresponding sections from the rat atlas in order to standardise the results across all experiments. The appearance of some histological sections was normal and the superimposed isopotential map then corresponded well with that superimposed onto the standard section (see figure 5.1).

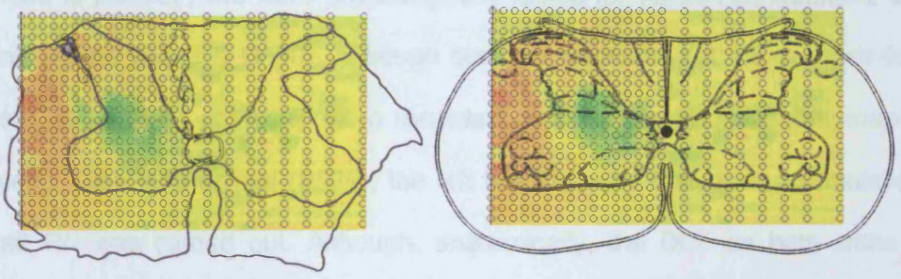


Figure 5.1 Isopotential map of R511
In this animal, the histological section was normal with hardly any distortion (A) and the isopotential map superimposed onto the standard rat atlas section (B) corresponded well with it. Arrows in A indicate marking electrodes which were used as landmarks for fitting the map

The isopotential maps presented in this thesis may not accurately reflect the exact location of synapses, but will indicate the presence of physiologically functional fibres, which anatomical tracing would not reflect. Therefore, this will allow the assessment of significant sprouting but could not be used for the finer detail such as exact location of synapses and which neurons the fibres terminate on. For this, the technique described here could be combined with others such as confocal microscopy. This method would also be ideal for the

assessment of plasticity and/or regeneration in other tracts where specific stimulation can be readily produced, for the example the CST.

5.4 Does the RST compensate for the loss of the CST?

It was reported here that spontaneous functional recovery, as assessed by the pellet retrieval test occurred following a lesion to the dorsal columns. However, if the lesion pathology extended to the DLF or the ventral spinal cord, success rate of the pellet retrieval test did not return to baseline levels. Of the three animals with a lesion that extended to the DLF, two were physiologically tested for the RN contralateral to the forelimb tested (R543 & R590). Although success rates did not return to pre-lesion levels, RST synaptic potentials were recorded, and a "normal" intraspinal map was produced. The third animal (R539), the left forelimb was tested, and stimulation of the left RN was carried out. Although, anatomically, the DLF on both sides was injured, but as in R543 and R590, the intraspinal map of field potentials produced was also "normal". The question that arises here, if in these three animals recovery to pre-lesion levels did not occur, but the synaptic potentials of the RST were relatively "normal", does the RST play a role in recovery? The RST is not the only pathway that runs in the DLF, other descending (lateral CST & the lateral reticulospinal tract) and ascending (spinocerebellar tract) pathways also run in the DLF.

Two studies on the involvement of the RST in pellet retrieval report conflicting results. Following a lesion to the RN, there was no effect on the success rate, and when the RN lesion was added to a previous lesion of the motor cortex, there was still no affect on the total number of reaches or the success rate (Whishaw *et al.*, 1990;Whishaw *et al.*, 1998). On the other hand, according to Schrimsher and Reier (1993), following a lesion to the RST, there was a significant reduction in the success rate. Although the

criteria for assessing success rates was slightly different (see behaviour discussion for details), the difference in results may still be attributed to damage of other pathways in the DLF.

As reported here, rubrospinal synaptic potentials of those animals with a lesion extending to the DLF appeared to be normal, and the observed reduction in success rate may be a result of the combined damage to the RST, the lateral CST, and the lateral reticulospinal tract. However, a combined lesion of the RST and the dorsal columns (excluding the CST) also resulted in a significant reduction in success rates with no return to pre-lesion levels (McKenna & Whishaw, 1999). Thus, it seems that a number of pathways are involved in the behaviour produced during pellet retrieval as deficits are seen following lesions to the dorsal columns, with, or without the CST, and the RST. Deficits are also seen following a lesion to the RN and the pyramids (for more details on lesion studies and references, see behaviour chapter). The information provided by the intraspinal maps of those animals with a lesion extending into the DLF clearly show that although the RST is still physiologically functional, it does not result in complete behavioural functional recovery, and therefore does not provide full compensation for damage to the CST.

5.5 No evidence of plasticity in the RST

From the anatomical and physiological studies of this thesis, there was no evidence of plasticity in the RST following a lesion to the dorsal columns. The spontaneous functional recovery of animals with a lesion to the dorsal columns cannot be attributed to sprouting from the RST. The development of an alternative reaching strategy suggests that the animals may not "recover" but instead learn to compensate with new movements to achieve their goal. When the lesion is extended

to include the DLF, and/or the ventral white matter, more components of the reaching are affected making it more difficult for the animals to successfully grasp the pellet. Compensatory movements are not enough to achieve success due to the increased deficits caused by damage to other pathways including the RST, the lateral and ventral CST, the vestibulospinal tract, and the reticulospinal tracts, and also ascending pathways. Significant sprouting of RST fibres has been shown to occur following therapeutic interventions, but not spontaneously (Raineteau *et al.*, 2002) and the experiments detailed in this thesis confirm this.

However, a different hypothesis was put forward by Pattersson (2000) following the observation that a small fraction of RST fibres enhance the speed of recovery of food taking in the cat. He suggested that when the command for food taking is passed via the remaining rubrospinal fibres, their activity will depolarise interneurons in the spinal network involved in food taking. This in turn facilitates the induction of plasticity in synapses of other systems on these interneurons. This hypothesis is based on that of associative plasticity. The reticulospinal system was put forward as a possible system involved in the takeover of command, as it is known that the rubrospinal and reticulospinal fibres converge on the same interneurons.

5.6 Implications and future work

The electrophysiological part of this study is the first neurophysiological study of the synaptic actions of the RST in the rat. The distribution of the synaptic activity of the RST of control animals and those with a cervical spinal cord injury has been reported in this thesis. This basic physiological data of the RST can be used by other investigators carrying out studies into the physiological activity of the RST, in particular if examined following an injury to the spinal cord. Following a spinal cord

injury (SCI), and in humans, some spontaneous recovery is often reported. However, the mechanisms that underlie this recovery are generally unknown. If functional recovery is seen in animal models of SCI, it is unknown if this recovery is due to plasticity or regeneration. And, if one or the other has been anatomically demonstrated, it is still not known if the fibres make functional connections. These questions are best addressed using physiological assessments. Thus, this thesis provides important data and protocols for the assessment of regeneration and plasticity in animal models of SCI. Using neurophysiological methods to assess connections of pathways before and after a spinal cord lesion will provide the crucial information required for the selection of pathways to be targeted for treatments and also in selecting therapeutic targets aimed at treating SCI in people.

The physiological data reported here for the RST will allow investigators to be aware of the models of RST stimulation, i.e. direct versus synaptic. As was reported, the late volley represents synaptic activation of the RST, and investigations of RST connectivity in control or lesioned animals could take this volley as an indication of RST activation. The early volley, although most often represents direct activation of the RST can sometime represent activation of other pathways. Also, activation of fibres from the RN contralateral to the stimulation site can occur resulting in synaptic responses in the ipsilateral spinal cord. These ipsilateral responses are especially important if assessing sprouting across the midline. Also, the reported labelling of fibres in the contralateral dorsal columns and in the ventral funiculus of the spinal cord must be taken into account, especially as this is not reported by other authors. Crossing fibres or fibres in the ventral horn may not be rubrospinal, but may be projections from the ventral funiculus. The physiological and anatomical observations of this thesis demonstrate that selective labelling or activation of the RST is difficult to achieve as shown by the non-RST labelling and the non-RST activation. All these

points reported in this thesis are crucial in assessing plasticity and regeneration in the RST.

The behavioural data together with the physiological data can be taken into account when assigning recovery to specific pathways in the spinal cord when only basic anatomical studies are used to assess pathways functionality. As shown in the current study, basic anatomical assessments of the lesion severity do not provide information on the exact extent of the lesion, as the physiological data demonstrated.

5.7 General conclusions

1. Following stimulation in and around the RN, two volleys were seen in the cord dorsum recording of the contralateral spinal cord, and one in the ipsilateral spinal cord, all with similar conduction velocities.

2. As previously reported in the cat and as seen here, the late volley represents synaptic activation of the RST, probably through the activation of fibres of the interpositus.

3. The early volley (contra- and ipsilaterally) most often represents direct activation of the RST, but may sometimes represent activation of fibres of other systems.

4. Synaptic responses of the RST were confined mainly to the intermediate grey of the contralateral spinal cord. Depending on the location of the stimulation electrode, synaptic potentials may also be seen in the ipsilateral spinal cord.

5. Anatomical tracing of the RST showed axon collaterals and terminals mainly within the intermediate grey of the contralateral spinal cord. Some RST fibres crossed the midline and others were seen projecting towards the most dorsolateral motor column, which were more common at C8 than C7.

6. As seen from the intraspinal maps of synaptic potentials, the focus of negativity extended towards the most dorsolateral motor columns in only one animal, in which the field potentials were recorded in C8. This corresponds to the anatomical observation of fibres in lamina IX mostly in C8.

7. Anatomical tracing of the RST provided support for the suggestion of somatotopy within the RN. In addition to this, an observation noted in the current study was that of a dense RST projection to the lateral part of the lamina IV and V, and also to the ventral part of lamina III.

8. As the cylinder test is best used for unilateral lesions, and there was no difference in sticker removal scores post-lesion compared to pre-lesion, the pellet retrieval test provided the most discriminatory information on the recovery of forelimb function post-lesion.

9. All animals (except R543), independent of lesion size, were able to successfully reach for the pellets across a 2cm gap. Those with no extension of lesion pathology to the DLF and/or ventral spinal cord recovered to pre-lesion success rate levels.

10. Following a lesion to the dorsal columns, animals used an alternative reaching strategy during the pellet retrieval test. All animals (except R608) demonstrated grasping and targeting deficits.

11. Physiological experiments added new information on the extent of the lesion in the spinal cord. Anatomical descriptions of the lesion did not provide information on the full extent of the lesion. CDP and field potential records indicated the extent of physiological damage to RST fibres. This may have an impact on the correlation of functional recovery to specific pathways in the spinal cord in which assessment of lesion size are carried out with using basic anatomical techniques.

12. From the anatomical and physiological data, there is no evidence of plasticity in the RST following a lesion to the dorsal columns including the CST.

Reference List

Alstermark B, Isa T, Ohki Y, & Saito Y (1999). Disynaptic pyramidal excitation in forelimb motoneurons mediated via C(3)-C(4) propriospinal neurons in the Macaca fuscata. *J Neurophysiol* **82**, 3580-3585.

Alstermark B, Isa T, Pettersson LG, & Sasaki S (2007). The C3-C4 propriospinal system in the cat and monkey: a spinal pre-motoneuronal centre for voluntary motor control. *Acta Physiol (Oxf)* **189**, 123-140.

Alstermark B, Lindstrom S, Lundberg A, & Sybirska E (1981a). Integration in descending motor pathways controlling the forelimb in the cat. 8. Ascending projection to the lateral reticular nucleus from C3-C4 propriospinal also projecting to forelimb motoneurones. *Exp Brain Res* **42**, 282-298.

Alstermark B, Lundberg A, Norrsell U, & Sybirska E (1981b). Integration in descending motor pathways controlling the forelimb in the cat. 9. Differential behavioural defects after spinal cord lesions interrupting defined pathways from higher centres to motoneurones. *Exp Brain Res* **42**, 299-318.

Alstermark B, Lundberg A, Pettersson LG, Tantisira B, & Walkowska M (1987). Motor recovery after serial spinal cord lesions of defined descending pathways in cats. *Neurosci Res* **5**, 68-73.

Alstermark B & Ogawa J (2004). In vivo recordings of bulbospinal excitation in adult mouse forelimb motoneurons. *J Neurophysiol* **92**, 1958-1962.

Alstermark B, Ogawa J, & Isa T (2004). Lack of monosynaptic corticomotoneuronal EPSPs in rats: disynaptic EPSPs mediated via reticulospinal neurons and polysynaptic EPSPs via segmental interneurons. *J Neurophysiol* **91**, 1832-1839.

Anderson KD, Gunawan A, & Steward O (2005). Quantitative assessment of forelimb motor function after cervical spinal cord injury in rats: relationship to the corticospinal tract. *Exp Neurol* **194**, 161-174.

Antal M, Sholomenko GN, Moschovakis AK, Storm-Mathisen J, Heizmann CW, & Hunziker W (1992). The termination pattern and postsynaptic targets of rubrospinal fibers in the rat spinal cord: a light and electron microscopic study. *J Comp Neurol* **325**, 22-37.

Baldissera F, Bruggencate GT, & Lundberg A (1971). Rubrospinal monosynaptic connexion with last-order interneurones of polysynaptic reflex paths. *Brain Res* **27**, 390-392.

Baldissera F, Hultborn H, & Illert M (1981). Integration in spinal neuronal systems. In *Handbook of physiology. The nervous system. Motor control* pp. 509-595. Bethesda, MD: Am physiol Soc.

Baldissera F, Lundberg A, & Udo M (1972). Stimulation of pre- and postsynaptic elements in the red nucleus. *Exp Brain Res* **15**, 151-167.

Ballermann M, McKenna J, & Whishaw IQ (2001). A grasp-related deficit in tactile discrimination following dorsal column lesion in the rat. *Brain Res Bull* **54**, 237-242.

Ballermann M, Tompkins G, & Whishaw IQ (2000). Skilled forelimb reaching for pasta guided by tactile input in the rat as measured by accuracy, spatial adjustments, and force. *Behav Brain Res* **109**, 49-57.

Bareyre FM, Kerschensteiner M, Raineteau O, Mettenleiter TC, Weinmann O, & Schwab ME (2004). The injured spinal cord spontaneously forms a new intraspinal circuit in adult rats. *Nat Neurosci* **7**, 269-277.

Barritt AW, Davies M, Marchand F, Hartley R, Grist J, Yip P, McMahon SB, & Bradbury EJ (2006a). Chondroitinase ABC promotes sprouting of intact and injured spinal systems after spinal cord injury. *J Neurosci* **26**, 10856-10867.

Barritt AW, Davies M, Marchand F, Hartley R, Grist J, Yip P, McMahon SB, & Bradbury EJ (2006b). Chondroitinase ABC promotes sprouting of intact and injured spinal systems after spinal cord injury. *J Neurosci* **26**, 10856-10867.

Bartholdi D & Schwab ME (1997). Expression of pro-inflammatory cytokine and chemokine mRNA upon experimental spinal cord injury in mouse: an in situ hybridization study. *Eur J Neurosci* **9**, 1422-1438.

Basso DM (2000). Neuroanatomical substrates of functional recovery after experimental spinal cord injury: implications of basic science research for human spinal cord injury. *Phys Ther* **80**, 808-817.

Beattie MS, Hermann GE, Rogers RC, & Bresnahan JC (2002). Cell death in models of spinal cord injury. *Prog Brain Res* **137**, 37-47.

Belhaj-Saif A & Cheney PD (2000). Plasticity in the distribution of the red nucleus output to forearm muscles after unilateral lesions of the pyramidal tract. *J Neurophysiol* **83**, 3147-3153.

Belhaj-Saif A, Karrer JH, & Cheney PD (1998). Distribution and characteristics of poststimulus effects in proximal and distal forelimb muscles from red nucleus in the monkey. *J Neurophysiol* **79**, 1777-1789.

Blagoveshchenskii ED, Pettersson LG, & Perfil'ev SN (2005). Control of fine movements mediated by propriospinal neurons. *Neurosci Behav Physiol* **35**, 299-304.

Blochlinger S, Weinmann O, Schwab ME, & Thallmair M (2001). Neuronal plasticity and formation of new synaptic contacts follow pyramidal lesions and neutralization of Nogo-A: a light and electron microscopic study in the pontine nuclei of adult rats. *J Comp Neurol* **433**, 426-436.

Bradbury EJ & McMahon SB (2006a). Spinal cord repair strategies: why do they work? *Nat Rev Neurosci* **7**, 644-653.

Bradbury EJ & McMahon SB (2006b). Spinal cord repair strategies: why do they work? *Nat Rev Neurosci* **7**, 644-653.

Bregman BS, Coumans JV, Dai HN, Kuhn PL, Lynskey J, McAtee M, & Sandhu F (2002). Transplants and neurotrophic factors increase regeneration and recovery of function after spinal cord injury. *Prog Brain Res* **137**, 257-273.

Bregman BS & Reier PJ (1986). Neural tissue transplants rescue axotomized rubrospinal cells from retrograde death. *J Comp Neurol* **244**, 86-95.

Brosamle C & Schwab ME (1997). Cells of origin, course, and termination patterns of the ventral, uncrossed component of the mature rat corticospinal tract. *J Comp Neurol* **386**, 293-303.

Brosamle C & Schwab ME (2000). Ipsilateral, ventral corticospinal tract of the adult rat: ultrastructure, myelination and synaptic connections. *J Neurocytol* **29**, 499-507.

Brown LT (1971). Projections and termination of the corticospinal tract in rodents. *Exp Brain Res* **13**, 432-450.

Brown LT (1974). Rubrospinal projections in the rat. *J Comp Neurol* **154**, 169-187.

Burke D (2001). Clinical relevance of the putative C-3-4 propriospinal system in humans. *Muscle Nerve* **24**, 1437-1439.

Canedo A (1997). Primary motor cortex influences on the descending and ascending systems. *Prog Neurobiol* **51**, 287-335.

Carlson SL, Parrish ME, Springer JE, Doty K, & Dossett L (1998). Acute inflammatory response in spinal cord following impact injury. *Exp Neurol* **151**, 77-88.

Caroni P, Savio T, & Schwab ME (1988). Central nervous system regeneration: oligodendrocytes and myelin as non-permissive substrates for neurite growth. *Prog Brain Res* **78**, 363-370.

Casale EJ, Light AR, & Rustioni A (1988). Direct projection of the corticospinal tract to the superficial laminae of the spinal cord in the rat. *J Comp Neurol* **278**, 275-286.

Castro AJ (1972). Motor performance in rats. The effects of pyramidal tract section. *Brain Res* **44**, 313-323.

Caughell KA & Flumerfelt BA (1977). The organisation of the cerebellorubral projection: an experiment study in the rat. *J Comp Neurol* **176**, 295-306.

Chambers WW & Liu CN (1957). Corticospinal tract of the cat: an attempt to correlate the pattern of degeneration with deficits in reflex activity following neocortical lesions. *J Comp Neurol* **108**, 23-55.

Cheema SS, Rustioni A, & Whitsel BL (1984). Light and electron microscopic evidence for a direct corticospinal projection to superficial laminae of the dorsal horn in cats and monkeys. *J Comp Neurol* **225**, 276-290.

Chen S & Aston-Jones G (1998). Axonal collateral-collateral transport of tract tracers in brain neurons: false anterograde labelling and useful tool. *Neuroscience* **82**, 1151-1163.

Chen Y, Chen XY, Jakeman LB, Chen L, Stokes BT, & Wolpaw JR (2006). Operant conditioning of H-reflex can correct a locomotor abnormality after spinal cord injury in rats. *J Neurosci* **26**, 12537-12543.

Cheney PD (1980). Response of rubromotoneuronal cells identified by spike-triggered averaging of EMG activity in awake monkeys. *Neurosci Lett* **17**, 137-142.

Coumans JV, Lin TT, Dai HN, MacArthur L, McAtee M, Nash C, & Bregman BS (2001). Axonal regeneration and functional recovery after complete spinal cord transection in rats by delayed treatment with transplants and neurotrophins. *J Neurosci* **21**, 9334-9344.

David S & Aguayo AJ (1985). Axonal regeneration after crush injury of rat central nervous system fibres innervating peripheral nerve grafts. *J Neurocytol* **14**, 1-12.

David S, Bouchard C, Tsatas O, & Giftochristos N (1990). Macrophages can modify the nonpermissive nature of the adult mammalian central nervous system. *Neuron* **5**, 463-469.

De Leon RD, Hodgson JA, Roy RR, & Edgerton VR (1998a). Full weight-bearing hindlimb standing following stand training in the adult spinal cat. *J Neurophysiol* **80**, 83-91.

De Leon RD, Hodgson JA, Roy RR, & Edgerton VR (1998b). Locomotor capacity attributable to step training versus spontaneous recovery after spinalization in adult cats. *J Neurophysiol* **79**, 1329-1340.

Diener PS & Bregman BS (1994). Neurotrophic factors prevent the death of CNS neurons after spinal cord lesions in newborn rats. *Neuroreport* **5**, 1913-1917.

Diener PS & Bregman BS (1998). Fetal spinal cord transplants support the development of target reaching and coordinated postural adjustments after neonatal cervical spinal cord injury. *J Neurosci* **18**, 763-778.

Dietz V (1995). Locomotor training in paraplegic patients. *Ann Neurol* **38**, 965.

Dietz V, Colombo G, Jensen L, & Baumgartner L (1995). Locomotor capacity of spinal cord in paraplegic patients. *Ann Neurol* **37**, 574-582.

Dusart I & Schwab ME (1994). Secondary cell death and the inflammatory reaction after dorsal hemisection of the rat spinal cord. *Eur J Neurosci* **6**, 712-724.

Edgerton VR, Leon RD, Harkema SJ, Hodgson JA, London N, Reinkensmeyer DJ, Roy RR, Talmadge RJ, Tillakaratne NJ, Timoszyk W, & Tobin A (2001). Retraining the injured spinal cord. *J Physiol* **533**, 15-22.

Edgerton VR & Roy RR (2002). Paralysis recovery in humans and model systems. *Curr Opin Neurobiol* **12**, 658-667.

Edgerton VR, Tillakaratne NJ, Bigbee AJ, de Leon RD, & Roy RR (2004). Plasticity of the spinal neural circuitry after injury. *Annu Rev Neurosci* **27**, 145-167.

Enríquez-Denton M, Toft A, & Riddell JS. An electrophysiological investigation of corticospinal actions in cervical segments of the rat spinal cord. J.Physiol 536.P, 53P. 2001. Ref Type: Abstract

Enríquez-Denton M, Toft A, & Riddell JS. Actions of the corticospinal tract in cervical segments of the rat spinal cord. soc.Neurosci.Abstr 363.6. 2002. Ref Type: Abstract

Fabes J, Anderson P, Yanez-Munoz RJ, Thrasher A, Brennan C, & Bolsover S (2006). Accumulation of the inhibitory receptor EphA4 may prevent regeneration of corticospinal tract axons following lesion. *Eur J Neurosci* **23**, 1721-1730.

Fanardjian VV, Papoyan EV, Pogossian VI, & Gevorkyan OV (1999). Comparison of the effects of electrolytic and chemical destruction of the red nucleus on the compensatory capacity of rats with rubrospinal tract lesions. *Neural Plast* **6**, 123-131.

Fanardzhyan VV, Gevorkyan OV, Mallina RK, Melik-Musyan AB, & Meliksetyan IB (2002). Dynamics of changes in operant reflexes in rats after transection of the corticospinal tract and removal of the sensorimotor region of the cerebral cortex. *Neurosci Behav Physiol* **32**, 477-484.

Fawcett JW & Asher RA (1999). The glial scar and central nervous system repair. *Brain Res Bull* **49**, 377-391.

Filbin MT (2003). Myelin-associated inhibitors of axonal regeneration in the adult mammalian CNS. *Nat Rev Neurosci* **4**, 703-713.

Fouad K, Schnell L, Bunge MB, Schwab ME, Liebscher T, & Pearse DD (2005). Combining Schwann cell bridges and olfactory-ensheathing glia grafts with chondroitinase promotes locomotor recovery after complete transection of the spinal cord. *J Neurosci* **25**, 1169-1178.

Friedman B & Aguayo AJ (1985). Injured neurons in the olfactory bulb of the adult rat grow axons along grafts of peripheral nerve. *J Neurosci* **5**, 1616-1625.

Fujito Y, Imai T, & Aoki M (1991). Monosynaptic excitation of motoneurons innervating forelimb muscles following stimulation of the red nucleus in cats. *Neurosci Lett* **127**, 137-140.

Futami T, Kano M, Sento S, & Shinoda Y (1986). Synaptic organization of the cerebello-thalamo-cerebral pathway in the cat. III. Cerebellar input to corticofugal neurons destined for different subcortical nuclei in areas 4 and 6. *Neurosci Res* **3**, 321-344.

Gibson AR, Houk JC, & Kohlerman NJ (1985). Magnocellular red nucleus activity during different types of limb movement in the macaque monkey. *J Physiol* **358**, 527-549.

Giesler GJ, Jr., Nahin RL, & Madsen AM (1984). Postsynaptic dorsal column pathway of the rat. I. Anatomical studies. *J Neurophysiol* **51**, 260-275.

Goldberger ME (1965). The extrapyramidal systems of the spinal cord: results of combined spinal and cortical lesions in the macaque. *J Comp Neurol* **124**, 161-174.

Gomez-Pinilla F, Ying Z, Roy RR, Molteni R, & Edgerton VR (2002). Voluntary exercise induces a BDNF-mediated mechanism that promotes neuroplasticity. *J Neurophysiol* **88**, 2187-2195.

Grillner S & Zangger P (1975). How detailed is the central pattern generation for locomotion? *Brain Res* **88**, 367-371.

Grillner S & Zangger P (1979). On the central generation of locomotion in the low spinal cat. *Exp Brain Res* **34**, 241-261.

Grimpe B & Silver J (2002). The extracellular matrix in axon regeneration. *Prog Brain Res* **137**, 333-349.

Gulino R, Dimartino M, Casabona A, Lombardo SA, & Perciavalle V (2007). Synaptic plasticity modulates the spontaneous recovery of locomotion after spinal cord hemisection. *Neurosci Res* **57**, 148-156.

Guth L, Zhang Z, & Steward O (1999). The unique histopathological responses of the injured spinal cord. Implications for neuroprotective therapy. *Ann N Y Acad Sci* **890**, 366-384.

Hendriks WT, Eggers R, Ruitenberg MJ, Blits B, Hamers FP, Verhaagen J, & Boe GJ (2006). Profound differences in spontaneous long-term functional recovery after defined spinal tract lesions in the rat. *J Neurotrauma* **23**, 18-35.

Hirschberg DL & Schwartz M (1995). Macrophage recruitment to acutely injured central nervous system is inhibited by a resident factor: a basis for an immune-brain barrier. *J Neuroimmunol* **61**, 89-96.

Holstege G (1987). Anatomical evidence for an ipsilateral rubrospinal pathway and for direct rubrospinal projections to motoneurons in the cat. *Neurosci Lett* **74**, 269-274.

Holstege G (1991). Descending motor pathways and the spinal motor system: limbic and non-limbic components. *Prog Brain Res* **87**, 307-421.

Holstege G, Blok BF, & Ralston DD (1988). Anatomical evidence for red nucleus projections to motoneuronal cell groups in the spinal cord of the monkey. *Neurosci Lett* **95**, 97-101.

Holstege G & Cowie RJ (1989). Projections from the rostral mesencephalic reticular formation to the spinal cord. An HRP and autoradiographical tracing study in the cat. *Exp Brain Res* **75**, 265-279.

Holstege G & Kuypers HG (1982). The anatomy of brain stem pathways to the spinal cord in cat. A labeled amino acid tracing study. *Prog Brain Res* **57**, 145-175.

Holstege G & Tan J (1988). Projections from the red nucleus and surrounding areas to the brainstem and spinal cord in the cat. An HRP and autoradiographical tracing study. *Behav Brain Res* **28**, 33-57.

Hongo T, Jankowska E, & Lundberg A (1965). Effects evoked from the rubrospinal tract in cats. *Experientia* **21**, 525-526.

Hongo T, Jankowska E, & Lundberg A (1969). The rubrospinal tract. I. Effects on alpha-motoneurones innervating hindlimb muscles in cats. *Exp Brain Res* **7**, 344-364.

Hongo T, Jankowska E, & Lundberg A (1972). The rubrospinal tract. IV. Effects on interneurones. *Exp Brain Res* **15**, 54-78.

Hongo T, Kitazawa S, Ohki Y, Sasaki M, & Xi MC (1989a). A physiological and morphological study of premotor interneurones in the cutaneous reflex pathways in cats. *Brain Res* **505**, 163-166.

Hongo T, Kitazawa S, Ohki Y, & Xi MC (1989b). Functional identification of last-order interneurones of skin reflex pathways in the cat forelimb segments. *Brain Res* **505**, 167-170.

Houk JC, Gibson AR, Harvey CF, Kennedy PR, & van Kan PL (1988). Activity of primate magnocellular red nucleus related to hand and finger movements. *Behav Brain Res* **28**, 201-206.

Houle JD & Ye JH (1999). Survival of chronically-injured neurons can be prolonged by treatment with neurotrophic factors. *Neuroscience* **94**, 929-936.

Huisman AM, Kuypers HG, & Verburgh CA (1981). Quantitative differences in collateralization of the descending spinal pathways from red nucleus and other brain stem cell groups in rat as

demonstrated with the multiple fluorescent retrograde tracer technique. *Brain Res* **209**, 271-286.

Humphrey DR, Gold R, & Reed DJ (1984). Sizes, laminar and topographic origins of cortical projections to the major divisions of the red nucleus in the monkey. *J Comp Neurol* **225**, 75-94.

Hyland B (1998). Neural activity related to reaching and grasping in rostral and caudal regions of rat motor cortex. *Behav Brain Res* **94**, 255-269.

Illert M, Lundberg A, Padel Y, & Tanaka R (1975a). Convergence on propriospinal neurones which may mediate disynaptic corticospinal excitation to forelimb motoneurones in the cat. *Brain Res* **93**, 530-534.

Illert M, Lundberg A, Padel Y, & Tanaka R (1978). Integration in descending motor pathways controlling the forelimb in the cat. 5. Properties of and monosynaptic excitatory convergence on C3--C4 propriospinal neurones. *Exp Brain Res* **33**, 101-130.

Illert M, Lundberg A, & Tanaka R (1975b). Integration in a disynaptic cortico-motoneuronal pathway to the forelimb in the cat. *Brain Res* **93**, 525-529.

Illert M, Lundberg A, & Tanaka R (1976a). Integration in descending motor pathways controlling the forelimb in the cat. 1. Pyramidal effects on motoneurones. *Exp Brain Res* **26**, 509-519.

Illert M, Lundberg A, & Tanaka R (1976b). Integration in descending motor pathways controlling the forelimb in the cat. 2. Convergence on neurones mediating disynaptic cortico-motoneuronal excitation. *Exp Brain Res* **26**, 521-540.

Illert M, Lundberg A, & Tanaka R (1977). Integration in descending motor pathways controlling the forelimb in the cat. 3. Convergence on propriospinal neurones transmitting disynaptic excitation from the corticospinal tract and other descending tracts. *Exp Brain Res* **29**, 323-346.

Illert M & Tanaka R (1978). Integration in descending motor pathways controlling the forelimb in the cat. 4. Corticospinal inhibition of forelimb motoneurones mediated by short propriospinal neurones. *Exp Brain Res* **31**, 131-141.

Isa T, Ohki Y, Seki K, & Alstermark B (2006). Properties of propriospinal neurons in the C3-C4 segments mediating disynaptic pyramidal excitation to forelimb motoneurons in the macaque monkey. *J Neurophysiol* **95**, 3674-3685.

Iwaniuk AN & Whishaw IQ (2000). On the origin of skilled forelimb movements. *Trends Neurosci* **23**, 372-376.

Jarratt H & Hyland B (1999). Neuronal activity in rat red nucleus during forelimb reach-to-grasp movements. *Neuroscience* **88**, 629-642.

Jeneskog T & Padel Y (1984). An excitatory pathway through dorsal columns to rubrospinal cells in the cat. *J Physiol* **353**, 355-373.

Jiang MC, Alheid GF, Nunzi MG, & Houk JC (2002). Cerebellar input to magnocellular neurons in the red nucleus of the mouse: synaptic analysis in horizontal brain slices incorporating cerebello-rubral pathways. *Neuroscience* **110**, 105-121.

Jin Y, Tessler A, Fischer I, & Houle JD (2000). Fibroblasts genetically modified to produce BDNF support regrowth of chronically injured serotonergic axons. *Neurorehabil Neural Repair* **14**, 311-317.

Keifer J & Houk JC (1994). Motor function of the cerebellorubrospinal system. *Physiol Rev* **74**, 509-542.

Keirstead SA, Vidal-Sanz M, Rasminsky M, Aguayo AJ, Levesque M, & So KF (1985). Responses to light of retinal neurons regenerating axons into peripheral nerve grafts in the rat. *Brain Res* **359**, 402-406.

Kennedy PR (1990). Corticospinal, rubrospinal and rubro-olivary projections: a unifying hypothesis. *Trends Neurosci* **13**, 474-479.

Kennedy PR & Humphrey DR (1987). The compensatory role of the parvocellular division of the red nucleus in operantly conditioned rats. *Neurosci Res* **5**, 39-62.

Keyvan-Fouladi N, Raisman G, & Li Y (2003). Functional repair of the corticospinal tract by delayed transplantation of olfactory ensheathing cells in adult rats. *J Neurosci* **23**, 9428-9434.

Keyvan-Fouladi N, Raisman G, & Li Y (2005). Delayed repair of corticospinal tract lesions as an assay for the effectiveness of transplantation of Schwann cells. *Glia* **51**, 306-311.

Kobayashi NR, Fan DP, Giehl KM, Bedard AM, Wiegand SJ, & Tetzlaff W (1997). BDNF and NT-4/5 prevent atrophy of rat rubrospinal neurons after cervical axotomy, stimulate GAP-43 and Talpha1-tubulin mRNA expression, and promote axonal regeneration. *J Neurosci* **17**, 9583-9595.

Koda M, Hashimoto M, Murakami M, Yoshinaga K, Ikeda O, Yamazaki M, Koshizuka S, Kamada T, Moriya H, Shirasawa H, Sakao S, & Ino H (2004). Adenovirus vector-mediated in vivo gene transfer of brain-derived neurotrophic factor (BDNF) promotes rubrospinal axonal regeneration and functional recovery after complete transection of the adult rat spinal cord. *J Neurotrauma* **21**, 329-337.

Kuchler M, Fouad K, Weinmann O, Schwab ME, & Raineteau O (2002). Red nucleus projections to distinct motor neuron pools in the rat spinal cord. *J Comp Neurol* **448**, 349-359.

Kuypers HG (1960). Central cortical projections to motor and somato-sensory cell groups. An experimental study in the rhesus monkey. *Brain* **83**, 161-184.

Kwon BK, Liu J, Messerer C, Kobayashi NR, McGraw J, Oschipok L, & Tetzlaff W (2002). Survival and regeneration of rubrospinal neurons 1 year after spinal cord injury. *Proc Natl Acad Sci U S A* **99**, 3246-3251.

Kwon BK, Liu J, Oschipok L, Teh J, Liu ZW, & Tetzlaff W (2004). Rubrospinal neurons fail to respond to brain-derived neurotrophic factor applied to the spinal cord injury site 2 months after cervical axotomy. *Exp Neurol* **189**, 45-57.

Lavrov I, Gerasimenko YP, Ichiyama RM, Courtine G, Zhong H, Roy RR, & Edgerton VR (2006). Plasticity of spinal cord reflexes after a complete transection in adult rats: relationship to stepping ability. *J Neurophysiol* **96**, 1699-1710.

Lawrence DG & Kuypers HG (1965). PYRAMIDAL AND NON-PYRAMIDAL PATHWAYS IN MONKEYS: ANATOMICAL AND FUNCTIONAL CORRELATION. *Science* **148**, 973-975.

Lawrence DG & Kuypers HG (1968a). The functional organization of the motor system in the monkey. I. The effects of bilateral pyramidal lesions. *Brain* **91**, 1-14.

Lawrence DG & Kuypers HG (1968b). The functional organization of the motor system in the monkey. II. The effects of lesions of the descending brain-stem pathways. *Brain* **91**, 15-36.

Lawrence DG, Porter R, & Redman SJ (1985). Corticomotoneuronal synapses in the monkey: light microscopic localization upon motoneurons of intrinsic muscles of the hand. *J Comp Neurol* **232**, 499-510.

Lawson LJ, Frost L, Risbridger J, Fearn S, & Perry VH (1994). Quantification of the mononuclear phagocyte response to Wallerian degeneration of the optic nerve. *J Neurocytol* **23**, 729-744.

Lemon RN. Pathways for corticospinal control of motoneurones in man and other primates. J.Physiol (London) 518P, 31S. 1999.
Ref Type: Abstract

Leong SK, Shieh JY, & Wong WC (1984). Localizing spinal-cord-projecting neurons in adult albino rats. *J Comp Neurol* **228**, 1-17.

Li S, Kim JE, Budel S, Hampton TG, & Strittmatter SM (2005). Transgenic inhibition of Nogo-66 receptor function allows axonal sprouting and improved locomotion after spinal injury. *Mol Cell Neurosci* **29**, 26-39.

Li S & Strittmatter SM (2003). Delayed systemic Nogo-66 receptor antagonist promotes recovery from spinal cord injury. *J Neurosci* **23**, 4219-4227.

Li Y, Field PM, & Raisman G (1997). Repair of adult rat corticospinal tract by transplants of olfactory ensheathing cells. *Science* **277**, 2000-2002.

Liang FY, Moret V, Wiesendanger M, & Rouiller EM (1991). Corticomotoneuronal connections in the rat: evidence from double-labeling of motoneurons and corticospinal axon arborizations. *J Comp Neurol* **311**, 356-366.

Liu Y, Himes BT, Murray M, Tessler A, & Fischer I (2002). Grafts of BDNF-producing fibroblasts rescue axotomized rubrospinal neurons and prevent their atrophy. *Exp Neurol* **178**, 150-164.

Liu Y, Kim D, Himes BT, Chow SY, Schallert T, Murray M, Tessler A, & Fischer I (1999). Transplants of fibroblasts genetically modified to express BDNF promote regeneration of adult rat rubrospinal axons and recovery of forelimb function. *J Neurosci* **19**, 4370-4387.

Lotan M, Solomon A, Ben Bassat S, & Schwartz M (1994). Cytokines modulate the inflammatory response and change permissiveness to neuronal adhesion in injured mammalian central nervous system. *Exp Neurol* **126**, 284-290.

Loy DN, Talbott JF, Onifer SM, Mills MD, Burke DA, Dennison JB, Fajardo LC, Magnuson DS, & Whittemore SR (2002). Both dorsal and ventral spinal cord pathways contribute to overground locomotion in the adult rat. *Exp Neurol* **177**, 575-580.

Lu P, Jones LL, Snyder EY, & Tuszynski MH (2003). Neural stem cells constitutively secrete neurotrophic factors and promote extensive host axonal growth after spinal cord injury. *Exp Neurol* **181**, 115-129.

Maier MA, Illert M, Kirkwood PA, Nielsen J, & Lemon RN (1998). Does a C3-C4 propriospinal system transmit corticospinal excitation in the primate? An investigation in the macaque monkey. *J Physiol* **511 (Pt 1)**, 191-212.

Martin GF & Fisher AM (1968). A further evaluation of the origin, the course and the termination of the opossum corticospinal tract. *J Neurol Sci* **7**, 177-187.

Massey JM, Hubscher CH, Wagoner MR, Decker JA, Amps J, Silver J, & Onifer SM (2006). Chondroitinase ABC digestion of the perineuronal net promotes functional collateral sprouting in the cuneate nucleus after cervical spinal cord injury. *J Neurosci* **26**, 4406-4414.

Massion J (1967). The mammalian red nucleus. *Physiol Rev* **47**, 383-436.

McCurdy ML, Hansma DI, Houk JC, & Gibson AR (1987). Selective projections from the cat red nucleus to digit motor neurons. *J Comp Neurol* **265**, 367-379.

McKenna JE, Prusky GT, & Whishaw IQ (2000). Cervical motoneuron topography reflects the proximodistal organization of muscles and movements of the rat forelimb: a retrograde carbocyanine dye analysis. *J Comp Neurol* **419**, 286-296.

McKenna JE & Whishaw IQ (1999). Complete compensation in skilled reaching success with associated impairments in limb synergies, after dorsal column lesion in the rat. *J Neurosci* **19**, 1885-1894.

Merrill EG & Ainsworth A (1972). Glass-coated platinum-plated tungsten microelectrodes. *Med Biol Eng* **10**, 662-672.

Metz GA & Whishaw IQ (2000). Skilled reaching an action pattern: stability in rat (Rattus norvegicus) grasping movements as a function of changing food pellet size. *Behav Brain Res* **116**, 111-122.

Mewes K & Cheney PD (1991). Facilitation and suppression of wrist and digit muscles from single rubromotoneuronal cells in the awake monkey. *J Neurophysiol* **66**, 1965-1977.

Mewes K & Cheney PD (1994). Primate rubromotoneuronal cells: parametric relations and contribution to wrist movement. *J Neurophysiol* **72**, 14-30.

Miller MW (1987). The origin of corticospinal projection neurons in rat. *Exp Brain Res* **67**, 339-351.

Muir GD & Webb AA (2000). Mini-review: assessment of behavioural recovery following spinal cord injury in rats. *Eur J Neurosci* **12**, 3079-3086.

Munz M, Rasminsky M, Aguayo AJ, Vidal-Sanz M, & Devor MG (1985). Functional activity of rat brainstem neurons regenerating axons along peripheral nerve grafts. *Brain Res* **340**, 115-125.

Nakajima K, Maier MA, Kirkwood PA, & Lemon RN (2000). Striking differences in transmission of corticospinal excitation to upper limb motoneurons in two primate species. *J Neurophysiol* **84**, 698-709.

Nathan PW & Smith MC (1982). The rubrospinal and central tegmental tracts in man. *Brain* **105**, 223-269.

Naus CG, Flumerfelt BA, & Hrycyshyn AW (1985). An HRP-TMB ultrastructural study of rubral afferents in the rat. *J Comp Neurol* **239**, 453-465.

Nicolas G, Marchand-Pauvert V, Burke D, & Pierrot-Deseilligny E (2001). Corticospinal excitation of presumed cervical propriospinal neurones and its reversal to inhibition in humans. *J Physiol* **533**, 903-919.

Nielsen JB, Perez MA, Oudega M, Enriquez-Denton M, & Aimonetti JM (2007). Evaluation of transcranial magnetic stimulation for investigating transmission in descending motor tracts in the rat. *Eur J Neurosci* **25**, 805-814.

Nudo RJ, Friel KM, & Delia SW (2000). Role of sensory deficits in motor impairments after injury to primary motor cortex. *Neuropharmacology* **39**, 733-742.

O'Leary DD & Terashima T (1988). Cortical axons branch to multiple subcortical targets by interstitial axon budding: implications for target recognition and "waiting periods". *Neuron* **1**, 901-910.

Onifer SM, Zhang YP, Burke DA, Brooks DL, Decker JA, McClure NJ, Floyd AR, Hall J, Proffitt BL, Shields CB, & Magnuson DS (2005). Adult rat forelimb dysfunction after dorsal cervical spinal cord injury. *Exp Neurol* **192**, 25-38.

Padel Y, Bourbonnais D, & Sybirska E (1986). A new pathway from primary afferents to the red nucleus. *Neurosci Lett* **64**, 75-80.

Padel Y & Jeneskog T (1981). Inhibition of rubro-spinal cells by somesthetic afferent activity. *Neurosci Lett* **21**, 177-182.

Padel Y, Rathelot JA, & Vinay L (1995). Analysis of potentials induced in red nucleus neurones from the somaesthetic pathway stimulated at the bulbar level. *Exp Brain Res* **106**, 377-390.

Padel Y & Relova JL (1988). A common somaesthetic pathway to red nucleus and motor cortex. *Behav Brain Res* **28**, 153-157.

Paxinos G & Watson C (1998). *The rat brain, in stereotaxic coordinates*, 4th ed.

Pearse DD, Lo TP, Jr., Cho KS, Lynch MP, Garg MS, Marcillo AE, Sanchez AR, Cruz Y, & Dietrich WD (2005). Histopathological and behavioral characterization of a novel cervical spinal cord displacement contusion injury in the rat. *J Neurotrauma* **22**, 680-702.

Perry VH, Brown MC, & Gordon S (1987). The macrophage response to central and peripheral nerve injury. A possible role for macrophages in regeneration. *J Exp Med* **165**, 1218-1223.

Pettersson LG, Blagovechtchenski E, Perfiliev S, Krasnochokova E, & Lundberg A (2000). Recovery of food-taking in cats after lesions of the corticospinal (complete) and rubrospinal (complete and incomplete) tracts. *Neurosci Res* **38**, 109-112.

Pierrot-Deseilligny E (1996). Transmission of the cortical command for human voluntary movement through cervical propriospinal premotoneurons. *Prog Neurobiol* **48**, 489-517.

Pompeiano O & Brodal A (1957). Experimental demonstration of a somatotopical origin of rubrospinal fibers in the cat. *J Comp Neurol* **108**, 225-251.

Priestley JV (2007). Promoting anatomical plasticity and recovery of function after traumatic injury to the central or peripheral nervous system. *Brain* **130**, 895-897.

Profyris C, Cheema SS, Zang D, Azari MF, Boyle K, & Petratos S (2004). Degenerative and regenerative mechanisms governing spinal cord injury. *Neurobiol Dis* **15**, 415-436.

Raineteau O, Fouad K, Bareyre FM, & Schwab ME (2002). Reorganization of descending motor tracts in the rat spinal cord. *Eur J Neurosci* **16**, 1761-1771.

Raineteau O, Fouad K, Noth P, Thallmair M, & Schwab ME (2001). Functional switch between motor tracts in the presence of the mAb IN-1 in the adult rat. *Proc Natl Acad Sci U S A* **98**, 6929-6934.

Raineteau O & Schwab ME (2001). Plasticity of motor systems after incomplete spinal cord injury. *Nat Rev Neurosci* **2**, 263-273.

Ramon y Cajal. Degeneration and regeneration of the nervous system Vol II. 542-516. 1928. Oxford university press.
Ref Type: Generic

Rathelot JA & Padel Y (1997). Ascending spinal influences on rubrospinal cells in the cat. *Exp Brain Res* **116**, 326-340.

Reid JM, Gwym DG, & Flumerfelt BA (1975). A cytoarchitectonic and Golgi study of the red nucleus in the rat. *J Comp Neurol* **162**, 337-361.

Reier PJ, Federoff S, & Vernadakis A. Astrocytes. 263-324. 1986. Academic press, New York.
Ref Type: Generic

Reier PJ, Stensaas LJ, Guth L, In Kao CC, & Bunge RP. Spinal cord reconstruction. 163-195. 1983. Raven Press, New York.
Ref Type: Generic

Reiner A, Veenman CL, Medina L, Jiao Y, Del Mar N, & Honig MG (2000). Pathway tracing using biotinylated dextran amines. *J Neurosci Methods* **103**, 23-37.

Relova JL & Padel Y (1989). Short latency somaesthetic responses in motor cortex, transmitted through the spino-thalamic system, in the cat. *Exp Brain Res* **75**, 639-643.

Robinson FR, Houk JC, & Gibson AR (1987). Limb specific connections of the cat magnocellular red nucleus. *J Comp Neurol* **257**, 553-577.

Rose PK, MacDonald J, & Abrahams VC (1991). Projections of the tectospinal tract to the upper cervical spinal cord of the cat: a study with the anterograde tracer PHA-L. *J Comp Neurol* **314**, 91-105.

Rossignol S (2006). Plasticity of connections underlying locomotor recovery after central and/or peripheral lesions in the adult mammals. *Philos Trans R Soc Lond B Biol Sci* **361**, 1647-1671.

Rouiller EM, Liang FY, Moret V, & Wiesendanger M (1991). Trajectory of redirected corticospinal axons after unilateral lesion of the sensorimotor cortex in neonatal rat; a phaseolus vulgaris-leucoagglutinin (PHA-L) tracing study. *Exp Neurol* **114**, 53-65.

Saling M, Sitarova T, Vejsada R, & Hnik P (1992). Reaching behavior in the rat: absence of forelimb peripheral input. *Physiol Behav* **51**, 1151-1156.

Schrimsher GW & Reier PJ (1992). Forelimb motor performance following cervical spinal cord contusion injury in the rat. *Exp Neurol* **117**, 287-298.

Schrimsher GW & Reier PJ (1993). Forelimb motor performance following dorsal column, dorsolateral funiculi, or ventrolateral funiculi lesions of the cervical spinal cord in the rat. *Exp Neurol* **120**, 264-276.

Sherrington CS (1910). Flexion-reflex of the limb, crossed extension-reflex, and reflex stepping and standing. *J Physiol* **40**, 28-121.

Shieh JY, Leong SK, & Wong WC (1983). Origin of the rubrospinal tract in neonatal, developing, and mature rats. *J Comp Neurol* **214**, 79-86.

So KF & Aguayo AJ (1985). Lengthy regrowth of cut axons from ganglion cells after peripheral nerve transplantation into the retina of adult rats. *Brain Res* **328**, 349-354.

Stewart DH, Preston JB, & Whitlock DG (1968). Spinal pathways mediating motor cortex evoked excitability changes in segmental motoneurons in pyramidal cats. *J Neurophysiol* **31**, 928-937.

Streit WJ, Semple-Rowland SL, Hurley SD, Miller RC, Popovich PG, & Stokes BT (1998). Cytokine mRNA profiles in contused spinal cord and axotomized facial nucleus suggest a beneficial role for inflammation and gliosis. *Exp Neurol* **152**, 74-87.

Tator CH (1995). Update on the pathophysiology and pathology of acute spinal cord injury. *Brain Pathol* **5**, 407-413.

Tator CH & Fehlings MG (1991). Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. *J Neurosurg* **75**, 15-26.

Terashima T (1995). Anatomy, development and lesion-induced plasticity of rodent corticospinal tract. *Neurosci Res* **22**, 139-161.

Thuret S, Moon LD, & Gage FH (2006). Therapeutic interventions after spinal cord injury. *Nat Rev Neurosci* **7**, 628-643.

Toyama K, Tsukahara N, & Udo M (1968). Nature of the cerebellar influences upon red nucleus neurones. *Experimental Brain Research* **4**, 292-309.

Tsukahara N, Fuller DR, & Brooks VB (1968). Collateral pyramidal influences on the corticorubrospinal system. *J Neurophysiol* **31**, 467-484.

Tsukahara N, Toyama K, & Kosaka K (1967). Electrical activity of red nucleus neurones investigated with intracellular microelectrodes. *Exp Brain Res* **4**, 18-33.

Vavrek R, Girgis J, Tetzlaff W, Hiebert GW, & Fouad K (2006). BDNF promotes connections of corticospinal neurons onto spared descending interneurons in spinal cord injured rats. *Brain* **129**, 1534-1545.

Webb AA & Muir GD (2003). Unilateral dorsal column and rubrospinal tract injuries affect overground locomotion in the unrestrained rat. *Eur J Neurosci* **18**, 412-422.

Webb AA & Muir GD (2004). Course of motor recovery following ventrolateral spinal cord injury in the rat. *Behav Brain Res* **155**, 55-65.

Weidner N, Ner A, Salimi N, & Tuszynski MH (2001). Spontaneous corticospinal axonal plasticity and functional recovery after adult central nervous system injury. *Proc Natl Acad Sci U S A* **98**, 3513-3518.

Wenk CA, Thallmair M, Kartje GL, & Schwab ME (1999). Increased corticofugal plasticity after unilateral cortical lesions combined with neutralization of the IN-1 antigen in adult rats. *J Comp Neurol* **410**, 143-157.

Whishaw IQ & Gorny B (1994). Arpeggio and fractionated digit movements used in prehension by rats. *Behav Brain Res* **60**, 15-24.

Whishaw IQ & Gorny B (1996). Does the red nucleus provide the tonic support against which fractionated movements occur? A study on forepaw movements used in skilled reaching by the rat. *Behav Brain Res* **74**, 79-90.

Whishaw IQ, Gorny B, & Sarna J (1998). Paw and limb use in skilled and spontaneous reaching after pyramidal tract, red nucleus and combined lesions in the rat: behavioral and anatomical dissociations. *Behav Brain Res* **93**, 167-183.

Whishaw IQ & Metz GA (2002). Absence of impairments or recovery mediated by the uncrossed pyramidal tract in the rat versus enduring deficits produced by the crossed pyramidal tract. *Behav Brain Res* **134**, 323-336.

Whishaw IQ & Pellis SM (1990). The structure of skilled forelimb reaching in the rat: a proximally driven movement with a single distal rotatory component. *Behav Brain Res* **41**, 49-59.

Whishaw IQ, Tomie JA, & Ladowsky RL (1990). Red nucleus lesions do not affect limb preference or use, but exacerbate the effects of motor cortex lesions on grasping in the rat. *Behav Brain Res* **40**, 131-144.

Wirz M, Zemon DH, Rupp R, Scheel A, Colombo G, Dietz V, & Hornby TG (2005). Effectiveness of automated locomotor training in patients with chronic incomplete spinal cord injury: a multicenter trial. *Arch Phys Med Rehabil* **86**, 672-680.

Wolpaw JR (2007). Spinal cord plasticity in acquisition and maintenance of motor skills. *Acta Physiol (Oxf)* **189**, 155-169.

Xiao M, Klueber KM, Lu C, Guo Z, Marshall CT, Wang H, & Roisen FJ (2005). Human adult olfactory neural progenitors rescue axotomized rodent rubrospinal neurons and promote functional recovery. *Exp Neurol* **194**, 12-30.

Xiao M, Klueber KM, Zhou J, Guo Z, Lu C, Wang H, & Roisen FJ (2007). Human adult olfactory neural progenitors promote axotomized rubrospinal tract axonal reinnervation and locomotor recovery. *Neurobiol Dis*.

Yang HW & Lemon RN (2003). An electron microscopic examination of the corticospinal projection to the cervical spinal cord in the rat: lack of evidence for cortico-motoneuronal synapses. *Exp Brain Res* **149**, 458-469.

Yasui Y, Yokota S, Ono K, & Tsumori T (2001). Projections from the red nucleus to the parvicellular reticular formation and the cervical spinal cord in the rat, with special reference to innervation by branching axons. *Brain Res* **923**, 187-192.

Ying Z, Roy RR, Edgerton VR, & Gomez-Pinilla F (2003). Voluntary exercise increases neurotrophin-3 and its receptor TrkC in the spinal cord. *Brain Res* **987**, 93-99.

Ying Z, Roy RR, Edgerton VR, & Gomez-Pinilla F (2005). Exercise restores levels of neurotrophins and synaptic plasticity following spinal cord injury. *Exp Neurol* **193**, 411-419.